A TOXICITY STUDY ON

"PAÑCACŪTA MEḶUKU"

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GOVT.SIDDHA MEDICAL COLLEGE, PALYAMKOTTAI

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **A Toxicity study on *Pañcacūta me̲luku*** is a bonafide and genuine research work carried out by me under the guidance of **Prof. Dr.M.Thiruthani M.D(s),** Post Graduate Department of Nanju Nooum Maruthuva Neethi Noolum, Govt Siddha Medical College, Palayamkottai and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or other similar title.

Date: **Signature of the Candidate**

Place: Palayamkottai

CERTIFICATE

This is to certify that the dissertation entitled **"A TOXICITY STUDY ON *PAÑCACŪTA MEḶUKU*"** is a bonafide work done by **Dr. R. Jeeva Nandhini (Reg.No. 321616002)** Govt. Siddha Medical College, Palayamkotai in partial fulfillment of the university rules and regulations for award for **MD(s) Nanju Noolum Maruthuva Neethi Noolum** under my guidance and supervision during the academic year 2016-2019.

Name and signature of the Guide:

Name and signature of the Head of Department:

Name and signature of the Principal:

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List of abbreviations

ANOVA	Analysis of variance
ALP	Alkaline Phosphatase Level
ALT	Alanine Transaminase
AST	Aspartate Amino Transferase
ATSDR	Agency for Toxic Substances and Disease Registry
CPCSEA	Committee for the Purpose of Control and Supervision of Experimental Animals.
FTIR	Fourier Transform Infrared Spectrometry
Hb	Haemoglobin
IAEC	Institutional Animal Ethical Committee
ICMR	Indian Council of Medical Research
ICPOES	Inductively Coupled Plasma Optical Emission Spectrometry
IMPCOPS	The Indian Medical Practitioner's Co-operative Pharmacy and Stores
OECD	Organization for economic corporation and development
PCV	Packed cell volume
PCM	Pancha Sootha Mezhugu
RBC	Red blood corpuscles
SEM	Scanning electron microscope
SEM	Standard error mean
SGOT	Serum Glutamic-oxaloacetic transaminase
SGPT	Serum glutamic Pyruvic transaminase
WHO	World Health Organization
WBC	White blood corpuscles
XRD	X-Ray Diffraction
XRF	X-Ray Fluorescence spectroscopy

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Note: All the Tamil terms in this work are transliterated in to roman numerals using ISO 1519 transliteration scheme and given in italics

List of transliterated words

eṇṇey	எண்ணெய்
ajīraṇak kaḻiccal	அஜீரண கழிச்சல்
akastiyar kuḻampu	அகஸ்தியர் குழம்பு
aḷavukku mīṟiṉāl amirtamum nañcu	அளவுக்கு மீறினால் அமிர்தமும் நஞ்சு
Aṇṭa tailam	அண்ட தைலம்
amirta veṇṇey	அமிர்த வெண்ணெய்
aṉupōka vaittiya navanītam	அனுபோக வைத்திய நவநீதம்
ariciyeṭai	அரிசி எடை
āṟumuka centūram	ஆறுமுக செந்தூரம்
aṣṭa pairavam	அஷ்ட பைரவம்
ayakānta centūram	அயகாந்த செந்தூரம்
ayavīra centūram	அயவீர செந்தூரம்
caṉṉi	சன்னி
caṇṭamāruta centūram	சண்டமாருதச் செந்தூரம்
carvāṅkavali	சர்வாங்க வலி
Cayittiyam	சயித்தியம்
cērāṅkoṭṭai tailaem	சேராங்கொட்டை தைலம்
cikiccai ratṉa tīpam	சிகிச்சா ரத்ன தீபம்
cīṉākkāram	சீனாக்காரம்
ciraṅku kaḷimpu	சிரங்கு களிம்பு
ciṟṟaṇṭa tailam	சிற்றண்ட தைலம்
cittira mūlam vēr	சித்திர மூலம் வேர்
civaṉār amirtam	சிவனார் அமிர்தம்
civappumeḻuku	சிவப்பு மெழுகு
Curam	சுரம்
curukku kuṭuttal	சுருக்கு கொடுத்தல்
cūṭaṉ	சூடன்
cuyamākkiṉi kumāri centūram	சுயமாக்கினி குமாரி செந்தூரம்
Icivu	இசிவு
iḷampiḷḷai vāyu	இளம்பிள்ளை வாயு
Iraca centūram	இரச செந்தூரம்
iraca kanti meḻuku	இரச கந்தி மெழுகு
iracakaṟpūram	இரச கற்பூரம்
Iracam	இரசம்
jalatōṣam	ஜலதோஷம்
jāti jampīra kuḻampu	ஜாதி ஜம்பீரக் குழம்பு
kāḷamēka nārāyaṇa centūram	காளமேக நாராயண செந்தூரம்
kalaṅku	களங்கு
kaḻaṟci tailam	கழற்சி தைலம்
kānta centūram	காந்த செந்தூரம்
kānta raca villai	காந்த ரச வில்லை
kantaka cuṭar tailam	கந்தக சுடர் தைலம்
karappāṉ tailam	கரப்பான் தைலம்
Karuppu	கரப்பான் தைலம்

karuvaṅka cuṉṉam	கருவங்க சுண்ணம்
kastūri karuppu	கஸ்தூரி கருப்பு
kāṭikāra centūram	காடிகாரச் செந்தூரம்
kaṭṭu	கட்டு
kauri cintāmaṇi centūram	கௌரி சிந்தாமணி செந்தூரம்
kōrōjaṉai māttirai	கோரோசணை மாத்திரை
kuḻampu	குழம்பு
kummaṭṭi meḻuku	கும்மட்டி மெழுகு
kuṭaiccal	குடைச்சல்
liṅkam	லிங்கம்
lōka maṇṭūra centūram	லோக மண்டூரச் செந்தூரம்
mahā vīra meḻuku	மகா வீர மெழுகு
Mai	ஐம
makappēṟṟukkuppiṉ caṉṉi	மகப்பேற்றுக்குப்பின் சன்னி
Mantam	மாந்தம்
maṇṭūra centūram	மண்டூரச் செந்
maṇṭūra centūram	மண்டூரச் செந்தூரம்
Māttirai	மாத்திரை
mēka viraṇa kaḻimpu	மேக விரண களிம்பு
meḻuku	மெழுகு
meḻuku	மெழுகு
muka vātam	முகவாதம்
muruṅkaippaṭṭai cāṟu	முரங்கை பட்டை சாறு
muruṅkaipū	முரங்கை பூ
naccu kaḻiccal	நச்சு கழிச்சல்
nāka paṟpam	நாக பற்பம்
nanti mai	நந்தி மை
nātakaru tailam	நாதகரு தைலம்
nava uppu meḻuku	நவ உப்பு மெழுகு
nellikkāy kantakam	நெல்லிக்காய் கந்தகம்
Neruñcilcamūlam	நெருஞ்சில் சமூலம்
Ōmam	ஓமம்
paccaikkaṟpūram	பச்சைக்கற்பூரம்
pāla cañcīvi māttirai	பால சஞ்சீவி மாத்திரை
pañca cūtam	பஞ்ச சூதம்
pañcacūta meḻuku	பஞ்ச சூத மெழுகு
paṟaṅkippaṭṭai pataṅkam	பறங்கிப்பட்டை பதங்கம்
paṟpam	பற்பம்
pāṣāṇa kuḻampu	பாஷாண குழம்பு
pāṭāṇam	பாடாணம்
pataṅkam	பதங்கம்
paṭṭu karuppu	பட்டு கருப்பு
pavaḷa vaṅka paṟpam	பவள வங்க பற்பம்
payiṟu piramāṇam	பயிறு பிரமாணம்
piṭippu	பிடிப்பு
pūnīṟu	பூநீறு
pūra kaṭṭu paṟpam	பூரக் கட்டு பற்பம்
pūra paṟpam	பூர பற்பம்

pūra poṭi	பூர பொடி
Pūram	பூரம்
pūrṉa cantirōtayam	பூர்ண சந்திரோதயம்
tātu carakkukaḷ	தாது சரக்குகள்
Tāyppāl	தாய்பால்
tirilōkac centūram	திரிலோக செந்தூரம்
tirimūrtti pataṅkam	திருமூர்த்தி பதங்கம்
tuvar eṇṇey	துவர் எண்ணெய்
Ulōkam	உலோகம்
Uparacam	உபரசம்
Uppu	உப்பு
uruttira pairavam	உருத்திர பைரவம்
vālai racam	வாலை இரசம்
vāṉ meḻuku	வான் மெழுகு
vaṅka cuṉṉam	வங்க சுண்ணம்
vaṅka viraṇa kaḷimpu	வங்க வீரண களிம்பு
vaṭṭu	வட்டு
vayiṟṟu uppucam	வயிற்றுப்பிசம்
veḷḷai pūṇṭu tailam	வெள்ளைப்பூண்டு தைலம்
veḷḷi paṟpam	வெள்ளி பற்பம்
veḷvaṅka paṟpam	வெள்வங்க பற்பம்
Vīram	வீரம்
viṣa pēti	விஷபேதி
viṣak kuḻampu	விஷக குழம்பு
yūki karical	யூகி கரிசல்

1 Introduction

Toxicity, the single word can alarm the whole population into fear. But in reality what does the word toxicity refer to? Dr.Jaising P Modi, the author of "A Textbook of Medical Jurisprudence and Toxicology" connotes toxicity as unwanted bad effects created by certain substances and it can be trivial to grave(1). In reality, almost all the substances from plants, animals, minerals, metals or any other sources will show toxicity above a certain threshold limit. A popular saying in Tamil — *aḷavukku mīṟiṉāl amirtamum nañcu* which means even elixir turns poisonous when taken in excess vouches this. This is also corroborated by descriptions of Paracelsus, a 16thcentuary physician, considered to be the first one to incorporate heavy metal-based drugs in western medical history. At present, a substance is deemed to be toxic (despite its proven medicinal values), if the threshold limit is very low and was always looked upon as a potential threat. For instance, some plants such as *Aconitum ferox*, seeds of *Datura sps, Strychnos nux-vomica, Abrus precatorius* etc and few metals such as Arsenic, Lead, Mercury, Cadmium etc. show a significant toxic effects even at very low doses. (2)Nevertheless, these so-called toxic substances have several centuries of usage for its medicinal values and contribute to the significant portion of all traditional medical systems (3)(4). However, after the dominance of western bio-medicine over other medical systems since the second half of 20thcentury, the righteous usage of all substances with toxic nature declined due to the reduction in number of traditional practitioners. Although, after the invention of various extraction and isolation techniques in later part of the 20thcentaury, the active ingredients were separately isolated from the potentially toxic ingredients in plants and medical usage of many plants with toxic nature has been regularized. But the medical use of metal-based substances is still debated due to the very limited understanding about the pharmacokinetics of these metals and frequent reports of toxicity associated with its

> All substances are poisons; there is none that is not a poison. The right dose differentiate a poison from a remedy
>
> - Paracelsus (16th century Physician, Father of Modern Toxicology)

usage. While there is no denying that these substances with low threshold toxic limit may become potentially fatal, its fabulous contribution in traditional healthcare should also be acknowledged. Most of the metals in general, and mercury in particular were of high concern in recent past for its usage in traditional medicine due to the various toxic reports associated with them(5).

When we look in to the historical usage of mercury, it has its strong roots in almost all the civilizations. It is best known for Egyptians and Greeks before 2500 BC and for Indian and Chinese civilization before 2000 BC(6). Since 500 BCE Greeks used inorganic mercury in the form of ointments to treat skin disorders (7)(7). Egyptians used cinnabar in cosmetics(8) and also for preserving the dead bodies as it delays decomposition of the body(9), resists insects and other microorganisms(10). Later, they started using this in paintings for its insect and microorganism repelling property to maintain the colour and texture of paintings for a long period (1). Chinese believed that mercury enhances the longevity (elixir) of their life, and to treat skin disorders(11). Apart from this they also used mercury as an aphrodisiac, sedative and contraceptive in women (12).

> *During 7th century Buddhist monk Nandi from central India, left to srilanka and south East Asia before arriving in China inA.D. 655. A year later, the emperor of China shipped him off to sea again to collect medicinal herbs. He returned to China in A.D. 663.....*
>
> *Nandi, also called as Näräyanasvämin, who was captured and held in the Chinese court in about A.D. 649 because he knew of an elixir of life. During664 AD, an Indian physician named Lokäditya were called to serve as an alchemist in residence. Through this process, A.D. 646, the Chinese offered a Sanskrit translation of the Tao teaching to the king of Kämarüpa (Assam), to exchange the knowledge on elixir alchemy*
>
> ***-The Alchemical Body***
> **- Prof. David Gordon White, 1996**

After the invention of barometer by Torricellinin 1643 and thermometer by Fahrenheit in 1720, mercury became inevitable part of research and medical field (13)(14)(6). In 17th century, Mercuric nitrate was adopted in belt and hat making process in France and followed by England, USA, Italy, Spain, Russia. Between 1760 and 1820, industrial revolution burst out and mercury demand has increased. This revolution has revamped the history of mercury usage and it is widely used as explosives and detonators in the form of mercury fulminate.In 1829, mercuric poisoning symptoms was observed largely among the Hatters, who were working in a Belt and Hat Manufacturing company, Russia.In the year 1860, an article was published regarding the mercury poisoning as “Mercurial disease among Hatters”(15). It explained the forms, usage and toxic effects of mercury to the loud.Apart from this,

gold amalgam (an alloy with mercury) thatwas widely used in gilding was found to be the cause for numerous casualties among the workers(16).

Since 1930, Thimerosal (form of ethyl mercury) was used as the most common preservative in vaccines, serums, anti-venoms, antigens, antibodies and bio products (17)(18). In 1956, most of the population in fisherman families in Minamata and Niigata bay of Japan were found to be affected by severe mercury poisoning characterized bysevere neurological defects. An industry manufacturing acetaldehyde using mercury catalyst located in Minamata that was dumping its industrial waste in to river beds was found to be the reason behind this tragedy(14). It took almost twelve years for the government to recognize the cause behind this tragedy.Following that WHO started creating awareness about methyl mercury consumption among the population. Almost 15 years after Minamata incident, in 1971 imported wheat seeds in Iraq caused a drastic mass poisoning among 7000 persons resulting in 500 mortalities (19).Upon investigation, methyl mercury used as a preservative for seed for of its strong fungicidal action was found to be major culprit. Twenty-five years later, in 1997, Karen E. Wetterhahn, a toxic metal specialist was accidentally poisoned by the dimethyl mercury due to unintended penetration into palms via her protective gloves. As the prevalence of occupational exposure gradually raised in later part of twentieth century, research for other alternatives for mercury-based products such as fluorescent bulb, battery etc. became popular (20)(21).

By the end of 20th century, due to various reports of occupational toxicity and environmental pollution, the direct usage and exposure of mercury was regulated in many countries(21). ATSDR (Agency for Toxic Substances and Disease Registry)an US based organization found in 1985, for the documentation of the individual toxicological profile of the metallic and chemical substances declare most of the metals as toxic and numbered mercury at number three next to lead and arsenic in ATSDR 2017 Toxic substance Priority List (22)(23). The Food and Drug Administration modernization Act of 1997, regulated the usage of certain products containing mercury including thimerosal(18). By the year 1999, the government sector amended that mercury containing products were restricted in manufacturing and some of the most important products were manufactured with certain modified formulation.

The roots of mercury usage in Indian sub-continent were very much evident from the works of Dr. Richard S Weiss and Prof. David Gordon White. In most of the instances, it’s been viewed as a substance of immortality and elixir of life.

When people today mention Bhogar, they almost always say that he traveled to China. Most ascribe antiquity to his texts, the most important being Pōkar 7000 . Layne Little points to features of the text, such as its references to technology, modern language, and even English words, that suggest its composition just before its initial publication in 1888. It describes technology such as steamboats, trains, parachutes, and telescopes. Bhogar travels to Rome via a kuëikai pill, discovers the "Well of Mercury," procures mercury after meditating on Manonmani and Shiva, and returns by flight to Potigai Mountain.

- Recipes of immortality
Dr. Richard S. Weiss, 2009

Indian medical systems in general and Siddha in particular advocates the use of mercury for almost all type of ailments, though it's been most often indicated for its aphrodisiac nature and its potential in management of skin and joint disorders. This system elaborates the individual action of *Pañcacūtam* and its toxic symptoms along with specific antidotes. After certain outbreaks of mercury, it has been using with stringent safety protocols as per WHO guidelines. Even though in the year 2004, Robert K Saper and team reported thatamong the 14 samples out of 70 Ayurvedic formulations purchased online (27 manufacturing companies) and showed higher levels of mercury, lead or arsenic than permissible limits. This created a huge impact in health policies of European countries resulting banning of the drugs listed by *Saper*(24).This created a fear among public in consumption of any herbo-metallic traditional drug. Hence in this study an attempt was made to have a complete contemporary understanding of a herbo-metallic drug *Pañca cūta meḻuku* (PCM).

1-A Aim and Objectives

Aim:

This study aims to evaluate the toxicity effect of *Pañca cūta meḻuku*. in experimental animals. The following objectives were followed in order to attain the aim of the study.

Primary objective:

- Preparation and Characterization of *Pañca cūta meḻuku*.
- Establish the safety profile and toxic effects of *Pañca cūta meḻuku.*

Secondary objective:

In order to attain the primary objectives, the secondary objectives were framed.

- Identification, collection and authentication of raw materials.
- Purification of raw materials and preparation of *Pañca cūta meḻuku*.
- Characterization of *Pañca cūta meḻuku* as per PLIM guidelines.
- Instrumentation analysis of PCM with FTIR, XRF and XRD.
- Evaluation of acute oral toxic effect of PCM in rat models as per OECD 425 guidelines.
- Evaluation of 28-days repeated oral toxicity of PCM in rat models as per OECD 407 guidelines.

2 Review of Literature

Siddha pharmacology (*kuṇapāṭam*) refers metal and minerals as *tātu carakkukaḷ* and classify them into 225 substances: 11 *ulōkam*: metals and its ores, 25 *uppu*: water-soluble salts that emit vapour on heating, 64 *pāṭāṇam*: water insoluble inorganic substances that emit vapour on heating, 5 *pañca cūtam*: mercury and its salts and 120 *uparacam*: water insoluble inorganic substances that do not emit vapour on heating. Classical *Siddha* texts describes about 64 drug variants among which 32 are internal and 32 are external(25).*Pañca cūtam* includes five forms of mercury: *iracam, liṅkam, pūram, vīram and iraca centūram* indicated in *Siddha* literatures for treatment of various ailments such as infectious diseases, rheumatic disorders, liver and respiratory disorders and in allergic conditions.There are many variants of *Pañca cūtam* based formulations in *Siddha* system such as *kaṭṭu, kalaṅku, vaṭṭu, centūram, paṛpam,meḻuku, kuḻampu, māttirai,*etc. (25)Few of them are listed in table 2.1. Each of these variants will have many formulations (E.g.: There are more than 50 different *centūram* prepared from *liṅkam* alone), which will result in infinite number of *Pañca cūtam* formulations.

Similarly, there are many different types of *Pañcacūtam* based formulations mentioned in various *Siddha* literatures and there is colossal difference between them in preparative procedures and ingredients involved in trituration. Some formulations include only trituration of *Pañcacūtams* with specific plant extracts followed by calcinations (26). Some formulations require multiple calcinations at each and every stage of trituration(25). In some formulations, apart from *Pañcacūtams*, many other metal/mineral ingredients were added along with different plant extracts for trituration followed by single or multiple calcinations(3). Depending on the nature of the procedures and total number of calcinations involved, it may take few months to few years to prepare a single variant of *Pañca cūtam*s based formulations. As there is no notable contemporary literature pertaining to the physio-chemical nature and biological activity any of these formulations is available, it is very difficult to understand the difference/similarity between these formulations.So exploring the science behind all *Pañcacūtam* based formulations in a single study is practically impossible. Hence PCM, a formulation that has all *Pañcacūtams* were considered for the present study.

Formulation variant	Name of the formulation	
Kaṭṭu	1. *Liṅka Kaṭṭu,* 2. *Vīra Kaṭṭu,*	3. *Pūra Kaṭṭu,* 4. *Iraca Kaṭṭu.*
Centūram	1. *Caṇṭamāruta Centūram* 2. *Maṇṭūra Centūram*	3. *Kāṭikkāra Centūram*
Mai	1. *Nanti mai*	
Paṟpam	1. *Veḷḷi paṟpam* 2. *Pūra Paṟpam*	
Karuppu	1. *Paṭṭu karuppu* 2. *Kastūri karuppu*	
Meḻuku	1. *Iraca kanti meḻuku* 2. *Mahā Vīra Meḻuku*	3. *Nava uppu meḻuku* 4. *Vāṉ meḻuku*
Kuḻampu	1. *Akastiyar kuḻampu* 2. *Jāti jampīra kuḻampu*	3. *Viṣak kuḻampu*
Māttirai	1. *Pāla cañcīvi māttirai* 2. *Gorojanai Māttirai*	3. *Uruttira pairavam* 4. *Aṣṭa pairavam*
Eṇṇey(Internal)	1. *Pūra Eṇṇey* 2. *Kantaka cuṭar tailam*	
Eṇṇey (External)	1. *TuvarEṇṇey* 2. *Karappāṉ tailam*	
Pataṅkam	1. *Paṟaṅkippaṭṭai Pataṅkam* 2. *Tirimūrtti Pataṅkam*	

A comprehensive literature search revealed few formulations similar to that of PCM. A formulation named '*Civappu meḻuku'* isprepared with*Pañcacūtams (iracam, liṅkam, pūram, vīram*and*iraca centūram), Ōmam* (*Carum copticum*), *cittiramūlam vēr* (root of *Plumbago zeylanica*), *cīṉākkāram* (*Alum*)and *pūnīṟu* (*Fuller's earth*) as major ingredients and is indicated for the treatment of *viṣapēti, carvāṅkavali, caṉṉi, jalatōṣam, cayittiyam, mantam, ajīraṇak kaḻiccal, vayiṟṟu uppucam* and*Icivu*. Even though,*Civappu meḻuku* has all forms of i*racam*, similar to that of PCM, the preparative protocol and indications are distinctively different from PCM. Apart from this, different variants of PCM could also be noted in Siddha texts.

Aṉupōkavaittiyanavanītam, part-7, describes a PCM formulation with*vīram, iraca karpūram, liṅkam, nellikkāy kantakam, iracam, muruṅkaippaṭṭai cāṟu, Tāyppāl, honey*as major ingredients. In this, instead of *iracacentūram,nellikkāy kantakam*is included and is indicated for form indicated for *naccu kaḻiccal (viṣa pēti), curam, caṉṉi* in dosage 1-2 *ariciyeṭai* (65-130 mg) with butter, palm jiggery as vehicle.

Aṉupōkavaittiya navanītam also describes another PCM formulation with same with purified *iracam, iraca karpūram, vīram, cūṭaṉ, paccaik kaṟpūram* and claims to cure*caṉṉi,cayittiyam, naccu kaḷiccal, makappēṟṟukkuppiṉ caṉṉi* when administered in 1-2 *ariciyeṭai* (65-130 mg)dosage with butter or palm jiggery as adjuvant. Here out of the five components of*pañcacūtam*, *iracacentūram* and *liṅkam* were replaced with *cūṭaṉ* (*Camphor*) and *paccaikkaṟpūram* (*Borneo camphor*). Moreover, none of the organic ingredients are included in this preparation.

A formulation in *cikiccairatṉatīpam* depicts *vaalairacam, liṅkam, pūram, vīram*and*iraca centūram* along with *cērāṅkoṭṭai tailam, veḷḷai pūṇṭu tailam* and *ciṟṟaṇṭa tailam* (3 *tailam* were taken together in equal quantity) as its ingredients. This PCM is specially indicated for various disorders ininfants and children: *curam*, *vayiṟṟu uppucam*, *kuṭaiccal*, *muka vātam*, *Icivu*, *piṭippu* and *iḷampiḷḷai vāyu* at a dosage of*payiṟu piramāṇam* (42 mg) with mother's milk as vehicle, twice a day for 3 days.

This should be administered with a strict food restriction. In case of infants, feeding mother should follow food restrictions. In case of adults, restriction should be based on severity of disease. If severity is high, tamarind should be restricted or else fried salt and fried tamarind should be advised.

Mercury-review:

Mercury exists in three oxidation states such as metallic, mercurous, mercuric form. Generally toxicity of mercury results by the presence of no. of mercuric ions binds to sulfhydryl groups of enzymes. In 1990, WHO limited the intake of inorganic mercury about 4µg/day. Some of the traditional system were used elemental and inorganic forms of mercury since the ancient such as Santeria, Espiritismo, Siddha and Ayurveda system(27). Mercury causes its toxic effect in neurological, renal, reproductive, immunological, respiratory and dermatological and also in developmental levels. The severity of toxicity depends on the chemical form and route of exposure. In general it mainly induces oxidative stress which eventually leads to enzymatic damage, membrane rupture and oxidation of biomolecules(28).

Metallic mercury:

Elemental or metallic mercury represented as Hg. In environment, mercury is released by various industrial activities such as pharmaceuticals, mining, industrial waste incineration and agriculture.

Source of exposure:

The main source of metallic mercury vapour exposure in humans is from occupation and dental amalgam (50% of metallic mercury)(29).

Uses:

Mainlyused as cathartics (accelerates defecation) and industrial products (thermometers, sphygmomanometer and batteries)(30).

Absorption:

It is poorly absorbed via gastrointestinal tract but 75-85% of vapour absorbed readily through mucus membrane to lungs especially causes damage to alveolar membrane and can passes blood brain barrier and placental barrier and cause damage to the target organs.(30)

Excretion takes place via sweat, saliva, urine and feces.

In the environment, the exposure of metallic Hg can persists for more than 4 years in air.

Action in human body:

Thehalf-life time of metallic mercury is 18 hours.

These ions in the blood stream transforms into inorganic mercury ions when it oxidized by catalase enzyme in tissues and this transformation limits the absorption of mercury into cell membrane as it is lipophilic in nature and readily diffusible into biological membrane and blood brain barrier. Brain is the target organ with oxidation reaction into divalent mercury.

Toxic symptoms:

It is reported that occupational exposure increase the concentration of mercury in blood to 18 nmol/L and on the other hand, the removal of dental amalgam makes the plasma concentration of mercury to range of 5 nmol/L.Through the dental amalgam fillings,

the released mercurial concentration ranges approximately 4-5 µg/day. Alsoit is reported that each amalgam releases 3-17 µgof mercury vapour per day.(29)Thus on comparing the occupational exposure, dental amalgam can exhibit long term exposure with trivial symptoms.

Its vapour causes temporary respiratory problems (bronchitis, asthma). It targets the tertiary and quaternary protein and disrupts the membrane potential and calcium homeostasis. At last free radicals were produced when the cell integrity is affected. Also intervenes in the process of transcription and translation. High level exposure causes lung damage, nausea, vomiting, diarrhoea, palpitation, skin rashes and increased blood pressure.

When once exposed to vapour, it deposits in brain with considerable amount and cause severe toxicity primarily in the brain and also induce severe pneumonia and sometimes fatal.Experimental studies revealed that occupational exposure affects central nervous system and the dental amalgam exhibits toxicity in kidney function.

Occupational exposure of elemental mercury leads to chronic poisoning with the following symptoms:

- Metallic taste in saliva
- Mucosal inflammation
- Gingivitis and stomatitis
- Nephrosis with albuminuria, hypoproteinaemia and edema
- CNS involvement induces
    - Tremor (bilateral intension tremor, initially in fingers, lips, eyes and progresses with painful spasms in extremities.
    - Mercurial erethism characterized by behaviour and personality changes with irritability, excitement, sleep disorder, depression and loss of self-control and confidence.
- General symptoms: weakness, tired, weight loss and anorexia.
- Repeated vapor exposure in children causes scarlet skin discoloration (pink disease/acrodynia) along with perspiration, tachycardia, increased blood pressure, sleeplessness and mood swings(31).

Some of the autopsy findings of dental amalgam bearers shows the presence of mercury about 2-12 fold in tissues than others. An animal experiment shows a significant

difference in concentration of mercury in brain as 10 times (7) increase than divalent mercury.

Inorganic mercury:

Inorganic mercury compounds are formed when it binds to some elements such as chlorine,sulphur or oxygen.These composition forms mercuric / mercurous salts such as mercuric chloride, Mercurous chloride,mercuric sulphide. Among those mercuric sulphide is also known to be cinnabar, which has been used since2000 years in Chinese traditional medicine and Indian traditional medicine (Siddha, Ayurveda).

Source of exposure:

Indirectly oxidation of mercury and demethylation of methyl mercury by intestinal micro flora sources the form of inorganic mercuric salts.(15)

Uses:

Inorganic mercury used in medicine preparation and in cosmetics preparation (soap, skin cream – 6-10% of mercuric chloride)(8)(7). Even it has been used in infant teeth powder, worm infestation drugs.

Mercurous chloride is also known to be calomel, was used as diuretics, antiseptics, skin ointments laxatives in medical history since ancient period.

Mercuric chloride holdsa history in medical system with its disinfectant and antiseptic activity.(5)

Absorption:

Ingested inorganic mercury shows 7-15 % of absorption through gastro intestinal tract.(30)

Sometimes these compounds were inhaled in the form of dust from the environment via lungs but this doesn’t shows any significant toxic difference.

Cinnabar is properly absorbed via gastro intestinal tract ranges from 0.1%-0.2% and has low bio- availability than mercuric chloride (10).These are less lipid soluble in nature and thus cant able to cross blood brain barrier and placental barrier. An experiment of external application in guinea pigs shows absorption of 2-3% of dose within 5 hours.

Excretion occurs through sweat, saliva, urine and feces.

Action in human body:

The half-life period of inorganic mercury is estimated to be 58 days.(32)According to brain it is estimated for several years.

Inorganic mercury can cause toxicity by means of oral administration and methyl mercury exposure.In human body,intestinal bacteria converts methyl mercury into inorganic mercuric salts and deposited in kidney.

Once enters the body, the mercurous ion dissociates into uncharged mercury Hg° and the mercuric ion.(17)

Toxic symptoms:

Toxic exposure of mercuric chloride is higher than mercurous chloride.

EPA and IARC declares that mercuric chloride as carcinogenic. Mercury poisoning is said to be acrodynia or pink disease(33). In general, increased exposure causes brain function alteration and few symptoms such as shyness, tremors, irritability, memory problems and visual and auditory disturbances.(30)

Effects on CVS:

Neurotoxicity is the primary effect caused by mercuric exposure and subsequently it produces cardio toxicity.(34)

Till 2000, the effect of mercury on cardio vascular system was unknown but minamata diseased patients suspected with myocardial infarction, coronary disease and other cardiovascular disease as their cause of death.(35)(36)(37)(38)

Acute toxic effects causes endothelial dysfunction and reduces nitric oxide and its bioavailability. The reduction of nitric oxide increases free radicals in the blood vessels. Chronic toxic effects causes severe endothelial damage and free radicals production in aorta, branches of mesenteric arteries and basilar arteries.

The main link observed on the effect of mercury on experimental animal shows between cardiovascular disease and hypertension with depressed myocardial contractility. But the toxic effect of mercury doesn't affect systolic pressure

Effect on Blood vessels:

In blood, initially it binds to sulfhydryl groups of erythrocytes and plasma proteins. It denature the proteins, inactivate enzymes and breakdown cell membranes (34)(38).

Recent year studies shows that it acts on the endothelium by reducing the nitric oxide and availability and thus increasing oxidative stress.

Atherosclerosis is the major effect caused by oxidative stress in vessels especially in aorta(28). Detailed study on vessels shows peripheral and pulmonary vasoconstriction.

Effect on endocrine receptors:

It causes significant reduction in T3 and T4 and increased TSH.

Effect on reproductive system:

Several animal experiments reported that inorganic form of mercury can develops testicular toxicity which includes reduction in spermatogenesis, induce androgen deficiency, alters the reproductive behaviour with oxidative stress (28)and lipid peroxidation of testicular membrane, affect leydig cells to reduce testosterone level and decreasing the LH (Luteinising hormone) and FSH (Follicular stimulating hormone).(39) But an experimental study with certain phyto therapeutic anti-oxidants shows significant reduction of toxic effects against mercury induced toxicity. Thereby Moringa oleifera reduces the lipid peroxidation in testicular membrane. (40)

Effects on CNS:

It induces neuronal degeneration and excitability which interacts with N-methyl-D-aspartate (NMDA) receptors.(13)

Because of neurotoxic compound, it cause damage to microtubules and mitochondria. It affects intracellular calcium homeostasis, alters protein phosphorylation, damages cell structure, interrupts the cell cycle and binds to cysteine or methionine rich proteins. In specific methyl mercury acts on neuron and inhibits the uptake of glutamate by astrocytes, leads to increased concentration of glutamate in extracellular fluid. Glutamate targets N-Methyl-D-Aspartate (NMDA) receptors and increases the level of sodium and calcium in neuronal cells.

Effect on Immune system:

It causes immune dysfunctions with hypersensitivity reactions (asthma, dermatitis, Tcell suppression, NK cell suppression and lymphocyte defect). Impaired immune system results in Kawasaki disease (31) which is characterized by photophobia, fever, pharyngitis, palpitation and Skin rashes.

Effects on Kidneys:

In kidney, heavy metals (mercury) primarily alters the permeability and absorption of epithelial cells results in proteinuria and kidney dysfunction.Nephrons plays a major role in active transport of mercury and other heavy metals. The route of exposure in renal cells is from luminal membranes in proximal tubule and sometimes through organic anion transporters of baso-lateral membrane.(41)

Chronic low dose exposure causes lesions in tubular, interstitial and glomerular parts. Inorganic chronic mercuric exposure targets Kidney with high concentration and 90% of absorption in proximal tubules of kidney and causes fibrotic changes in glomeruli, deposition of Ig G, IgM and C3 in basement membrane of glomerular. (30)

Oxidative stress is the important mechanism in cellular damage. An experiment in male Sprague-Dawley rats shows a significant reduction in the activities of certain antioxidant enzymes in the renal cortex such as superoxide dismutase, catalase, GSH peroxidase and GSH disulphide. Also oxidative stress can cause damages in glomeruli, interstitial tubule and renal vasculature.(28)

It can also leads to acute tubular necrosis, immunological glomerulonephritis, nephrotic syndrome.

Effects on GIT:

The toxic symptoms of inorganic salt occurs in two different phases. First phase occurs soon after the ingestion, the corrosive nature of salts causes burning sensation in the chest, discolouration of mucous membranes, gastro intestinal pain and sometimes vomiting, profuse bloody diarrhoea can lead to hypovolemic shock and death. It causes severe enterocyte protein precipitation in gut mucosa.

Inorganic salts have been commonly used in medicine especially calomel as purgative in the form of ‘blue pill’. Though calomel has low solubility and significantly produces less toxicity than mercuric chloride.

Organic mercury:

Organic Mercury also called as organometallic compound which results from the organic functional group such as Methyl mercury, Ethyl methyl or Phenyl group.

Source of exposure:

mercuric ions in the environment by microorganisms present in soil and water.The aquatic microorganisms with The common form of organic Hg is methyl and is formed by methylation of inorganic methylated Hg is the only source of prey for certain small fishes (Tura, shark) and they were consumed by large fishes and finally humans were tempted to have fish as their protein diet.(42) Sometimes if methylmercury exists in nature, it may be from methylation of inorganic mercury by aquatic sulphatereducing anaerobic bacteria.(32)

Uses:

Since the ancient it was used as antiseptics, diuretics and also pesticides .Since 1930, Thimerosal an organic mercurial preservative to prevent microbial contamination in vaccines and serums.(18)

History events:

Thimerosal:

Ethyl mercury, the most used preservative as Thimerosal since 1930 in vaccines and bio products. Approximately, it weighs with 50% of mercury and widely used to avoid microbial contamination in children vaccines, serums and all biological drugs.It is reported that a vaccine contains 0.01%thimerosal which means to be 50 micrograms of mercury approximately per 0.5 ml dose.

The public Health Services (including the FDA, National Institute of Health (NIH), CDC and so..,) reviewed the usage and effects of thimerosal in children's which results that there was no health issue but they decided to minimize the cumulative exposure of mercury in children to avoid the cumulative toxicity exposure of mercury in lifetime.

After the invention of Thimerosal, subjected to numerous elaborate studies and finally implement with high effectiveness in preventing contamination of bacteria and fungi in vaccines and cause no ill effects.

Minamata disease:

In japan, mercury in the industrial wastes dissolves into minamata Bay and cause severe toxicity in the year 1956.After that incident, the methyl mercury toxicity is also named to be minamata disease.Ecosystem plays a predominant role in developing toxicity in humans.

Once, it dissolves into Bay water, the aquatic microorganisms (certain Bacteria) consumes the industrial waste along with Hg and then the inorganic mercuric salts intoorganic mercury by methylation process.

Industrial waste ➡ Aquatic microorganisms ➡ fish ➡ Human ➡ Toxic Symptoms

At the time of minamata disease outbreak, approximately 2252 patients were diagnosed, 1043 were died of minamata disease.

The aetiology was not known until the death of cats ensured with similar symptoms equivalent to the symptoms of methyl mercury administration at a range of 1mg/kg of cat's body weight and also certain organs such as brain, liver, kidneys and hair were noted with high concentration of deposition.

Absorption& excretion:

About 95% methyl mercury absorption takes place via gastro intestinal tract and within 30 hours.

90% excretion occurs through bile and feces, 10% in urine.Once it is secreted in bile, it completes the entero-hepatic cycle when reabsorbs into blood stream.Its elimination undergoes first order Kinetics.

Action in human body:

This can easily pass through biological membranes, blood brain barrier and placental barrier since they are lipophilic in nature.(43)

The half-life of ethyl mercury is about one-third longer than methyl mercury (about 70 days).(32)Once it enters blood stream, reacts with catalase and sulfhydryl group and distributed in heart, liver,kidney, brain, placenta, foetus especially in peripheral nerves and bone marrow.

Approximately thimerosal weight with 50% of mercury and widely used to avoid microbial contamination in children, serums and all biological drugs. In the body, it metabolises into ethyl mercury and thiosalicylate and readily eliminated and may sometimes with mild toxicity but yet not reported. Ethylmercury converts into inorganic mercury and causes damages to brain cells.

In animal experiments the compound methyl mercury causes neurological alterations with increased reactive oxygen species.

Toxic symptoms:

Organic mercuric poisoning causes depression, memory impairment, tremor, fatigue, head ache, hair loss, etc. Methyl mercury is declared to be carcinogenic by EPA and IARC. Since it is a neurotoxic compound, it causes mitochondrial damage, microtubule damage and lipid peroxidation and finally it gives way to serotonin, glutamate and aspartate accumulation.

Immediate within 3 months of exposure, patients were diagnosed with acute and sub-acute poisoning, reported with symptoms such as constriction of vision, Ataxia, tremor, dysarthria with auditory and sensory disturbances.

Pathologically, it is characterized with cell damage in cerebral and cerebellar cortex, vision centre, auditory centre,pre and post central cortex (Sensory and motor centre) and noted that atrophy of cerebellum and demyelination of nerve fibres (20). And certain symptoms occurs after the onset of acute poisoning such as hypertension, hypersalivation, paraoxysmal symptoms, pyramidal symptoms and strabismus.

In 1959, a previousstudy report elucidated the concentration of mercury of organs such as liver, brain and kidney approximately ranges 22.0 to 70.5 ppm, 2.6 to 24.8 ppm and21.2 to 14.00 ppm respectively in persons who were died in minamata outbreak.

The exposure of methyl mercury in infants causes cerebral palsy and its concentration in umbilical cord seems high level which develops congenital minamata disease with atrophy and hypoplasia of brain cortex.

A most important high toxic organic mercury is considered to be dimethyl mercury, used for chemical analysis in the laboratories. After an incident that few drops of dimethyl mercury penetrates through the gloves to toxic metal specialist in the year 1997.Until six months from the time of exposure, it doesn’t shows any symptoms and followed sever toxicity, coma and death. Then the use in laboratories are strictly banned.

The toxic effect of methyl mercury targets central nervous system substantially the developing brain than the mature organs. Its latent period varies depending on the dose and period of exposure. Paraesthesia is the initial symptom of low dose exposure and progresses to cerebellar ataxia which results due to the loss of granular cells.(30)

Until 1970 methyl mercury and ethyl mercury were used for multiple purposes. After the issue of Iraq mass poisoning with 500’s of death by the usage of wheat grains which

preserved with fungicide (methyl mercury and ethyl mercury exhibits anti-fungal activity), the both were banned from usages.(19)

The US Environmental Protection Agencies endorsed a reference value of mercury concentration in blood (5.8 ng/ml) and WHO recommends a reference range of mercury concentration in hair (<6 µg/g **)** and considered below this level mean to be safe.

Some reports showed that there is increased concentration of mercury in hair which reaches 150 ug/g for the people who were inhabited in coastal areas where, the main source of dietary protein is fish.

It deposits in maximum concentration in almost all tissues of the body, approximately 5% in blood and 10% in brain.

On viewing the concentration in blood compartment, the red blood cells carries 20 times than plasma .Also Methyl mercury can able to crosses the blood brain barrier and the placental barrier.Thus the concentration in foetal brain is 5-7 times than that of maternal blood.And exposure can cause accumulates in growing scalp hair.The most common biological indicator for the exposure of mercury is hair and blood.

Mercury sulphide:

It is also known to be Cinnabar.Cinnabar is the only form of inorganic form which has been used since2000 years ago in medicine.Mercury sulphide is a form of divalent mercury and follows the mechanisms of distribution and metabolism but there is a significant difference in absorption and bioavailability.(44)The rate of absorption from gastro intestinal tract is estimated to be 0.2% and it deposition in tissues is approximately 10-100 fold less than mercuric chloride. On comparing mercuric chloride, the bioavailability decreases with 30-60 fold and thus is has been used high in treating diseases since the past in traditional system of medicine. (10)

Still cinnabar has been used in Traditional Chinese medicine with 11-13% range in the treatment of insomnia, anxiety and depression but it seems to be 110,000 times higher than the allowable dose recommended by European Drug and Food Standards.(45)

Mercury poisoning:

It occurs in two phases as acute and chronic. The generally mercuric ion binds with sulfhydryl group of enzymes and cellular proteins, nucleic acids and mitotic

apparatus interfering with enzyme and cellular transport functions. It is rapidly converted into mercuric ions leads to renal tubular damage. (41)

Acute poisoning:

Acute exposure toelemental mercury vapour may cause corrosive bronchitis with fever, chills and dyspnoea. It may progress to pulmonary oedema and fibrosis. Sometimes clinical features similar to Kawasaki disease (mucocutaneous lymph node syndrome) especially in children.

Symptoms:-

Acrid metallic taste, constriction in the throat, hoarse voice, mouth and tongue shows greyish white coating, vomit contains greyish slimy mucoid material with blood and shreds of mucous membrane. Circulatory collapse occurs. If survives, sometimes symptoms extend after 3 days such as glossitis, ulcerative gingivitis, loosening of teeth and necrosis of jaw. In 2-3 days renal tubular necrosis with polyuria, albuminuria, uraemia and acidosis. It produces dysentery ulceration with colonic mucosal hemmorhage.

Chronic poisoning:

Chronic poisoning of mercury is known to be Hydrargyrism. This may results from continuous accidental absortion, excessive therapeutic use, recovery from a large dose, externally used for a long time.

Symptoms:

It leads to classic triad of gingivitis and salivation, tremors and neuropsychartic changes. Earlier symptoms are blur line at the junction of teeth, gum inflammation, gastrointestinal disturbances, loss of weight and chronic kidney inflammation with progressive uraemia. Tremors called to be Danbury tremors occurs first in hands and progress to lips and tongue and finally to legs and arms. In advanced condition known as hatters shakes or glass-blowers shakes. The most severe form is concussio mercurialis, a condition when no activity is possible. Renal damage results in membranous glomerulonephritis with fatty cast in urine.

Mercurial erethism is seen in mirror manufacturing units which includes anxiety, depression, timidity, delusions, hallucinations or suicidal melancholia or manic

depressive pshychosis (mad hatter), emotional instability, insomnia and loss of memory.

Mercuria lentis is a peculiar eye change due to exposure to vapour of mercury. Due to brownish deposit of mercury through the cornea on the anterior lens capsule.

Acrodynia or pink disease (generalised body rash) thought to be an idiosyncratic hypersensitivity reaction particularly in children. The onset is insidious with anorexia, insomnia, skin rash, photophobia, sweating, paraesthetic with peeling of skin.

General treatment protocol:

- Removal of patient from further exposure
- Demulcents
- Saline purgatives
- Oral hygiene
- Chelation therapy (D-Penicillamine)
- For organic mercurial chelation is not effective.

Even though most of the researches were done in modern period, an ancient system of medicine called Siddha system of medicine eventually elaborates a substance in all aspects such as character, medicinal properties, growth, toxicological, adverse effect and the antidote. Especially mercury and its compounds were illustrated with a specific name 'panchasootham' which includes mercury, mercurous chloride, mercuric chloride, mercury suphide (artificial) and mercury sulphide (natural).the characters and medicinal properties with toxicological symptoms are described below.

The most specific names of rasam which is repeatedly used since ancient to till date are:

"ஆறியே சூதமது ஐந்து விதமாகும்
அதன் விபரமே தென்னிலறையைக்கேளு
ஊறியே ரசமென்றும் ரசேந்திரமென்றும்
உற்ற பாரத மென்றுஞ் சூதமென்றும்
மீறியே மிசிரகமென்றைந்து மாச்சு"

- போகர் 7000 2ம்காண்டம்

1. இரசம்
2. இரசேந்திரன்
3. சூதம்

4. மிசரகம்
5. பாரம்

ஐவித ரசத்தின் பண்பு (General properties of five forms of mercury):

"தங்கியதோர் ரசத்தினுடை வண்ணங்கேளு
தனியிரத்த நிறமாகத் தளுக்குத் தானாய்
மங்கியதோர் தோடமற்று ரசாயனமுமாகி
----------"

இரசம், பாரதம், சீவன்விந்து எனப்படும் இத்தாது பாடாண தொகுப்பில் பிறப்பு பாடாணமாகவும், வைப்பு பாடாணமாகவும் கூறப்பட்டுள்ளது. (போகர் 7000)

The above quote explains that each form of mercury shows a uniqueness in their colour, texture and character.

சுவை : அறுசுவை (இனிப்பு)

வீரியம் : வெப்பசீதம்

பிரிவு : **துணைமருந்தின் பிரிவு**

செய்கை : உடல்தேற்றி, உடல் உரமாக்கி, மலம்போக்கி, பித்தநீர் அகற்றி, வீக்கமுருக்கி, உமிழ்நீர் பெருக்கி, சிறுநீர் பெருக்கி, மேகநாசனி

Even though each of them were contaminated with other metals and some substances by source. So purification is considered to be the essential part prior to medicine preparation. And the Siddha system of medicine elucidates the contaminants of merury briefly in Gunapadam Thathu-Jeevam literature.

The contaminants and its remedy as per Siddha literature (தோடங்கள்):

தோடங்களை இரசத்தின் நஞ்சு தோஷங்களாக கருதுகின்றனர். இதில் சட்டைகள் (கவசம்) என்று ஒருவகையும் உள்ளது. இரசத்தின் தோடம் எண் வகையாகவும், இரசத்தின் சட்டை எழு வகையாகவும் விளக்கியுள்ளனர்.

தேரையர் கூறும் எண்வகைத் தோடங்களும் அதன் நோய்களும்

1.	சர்ப்பம்	-	கொப்பளம்
2.	வங்கம்	-	குட்டம்
3.	கந்தி	-	அக்கினி
4.	வன்னி	-	எரிச்சல்
5.	சாஞ்சலம்	-	கோதுநிறம்
6.	மலம்	-	விந்துநட்டம்
7.	காளம்	-	இறத்தல்
8.	மந்தம்	-	மூர்ச்சை

அகத்தியர் கூறும் ஏழு தோஷங்களும் அதன் நோய்களும்

1. நாகம் - துர்க்கந்தம்
2. வங்கம் - குட்டம்
3. கந்தி - தாகம்
4. வன்னி - தாது நட்டம்
5. சாஞ்சலம் - உயிர்போக்கல்
6. விடமம் - விடப்பிணி
7. லோகம் - மோகம்

அகத்தியர் மற்றும் தேரையர் கூற்றிணை ஒப்பிட்டு பார்க்க, 4 தோஷங்கள் ஒற்றுமையாக கூறப்பட்டு விளக்கியுள்ளனர். அவற்றிலும் அதற்காக கூறி போந்த நோய் நிலை மாறுபட்டபோதிலும் வங்கத்தின் தோடம் நிலையான நோய் நிலை கொண்டு விளக்கப்படுகிறது.

இது மட்டுமல்லாது கீழ்காணும் சில நூற்களிலும் தோடங்கள் பற்றி விவரித்துள்ளனர்.

1. தேரையர் கரிசல் 300
2. போகர் 7000
3. திருமூலர் 600

அவற்றை நீக்கும் முறைமை பற்றியும் உரைத்துள்ளனர்.(remedy to remove the contaminants):

1. நாகம்: இரச எடைக்குப் பதினாறெடை, செங்கல்தூள், மஞ்சள் தூள் இவைகளை ஒன்றன்பின் ஒன்றாகப் போட்டு, எலுமிச்சம் பழச்சாற்றால் ஒருநாள் அரைத்துப் புளித்த தண்ணீரில் கழுவினால் நாக தோடம் நீங்கும்.
2. வங்கம்: விசாலம் (கடம்பு) அழிஞ்சில் வேர் போட்டுப் பழச்சாற்றால் ஒரு நாள் அரைத்துக் காடிநீரால் கழுவ வேண்டும்.
3. மலம்: இலந்தைவேர் சேர்த்துப் பழரசத்தால் ஒரு நாள் அரைத்துக் காடி நீரால் கழுவ வேண்டும்.
4. அக்கினி: சித்திர மூலப்பட்டைச் சேர்த்துப் பழரசத்தால் அரைத்துக் காடியினால் கழுவவேண்டும்.
5. சாஞ்சல்யம்: கரூவூமத்தை வேர் சேர்த்துப் பழரசத்தால் அரைத்து காடியினால் கழுவ வேண்டும்.
6. விடம்: திரிபலைச் சூரணம் சேர்த்துப் பழச் சாற்றினால் அரைத்துக் காடியினால் கழுவ வேண்டும்.
7. கிரி: திரிகடுகுச் சூரணஞ் சேர்த்துப் பழச்சாற்றால் அரைத்துக் காடியினால் கழுவ வேண்டும்.
8. அசக்யாக்னி: நெருஞ்சில் சூரணம் சேர்த்துப் பழச்சாற்றால் அரைத்துக் காடியினால் கழுவவேண்டும்.

பாதரசத்தின் பொதுக்குணம்

"விழிநோய் கிரந்திகுன்மம் மெய்ச்சூலை புண்குட்
டழிகாலில் விந்துவினால் அத்தை – வழியாய்
புரியு விதி யாது புரியினோ யெல்லாம்
இரியு விதி யாது மில்லை"

சிவவீரிய மென்கிற இரசத்தை, முறைப்படி மருந்தாக்கிக் கொடுக்க, அது கண்நோய், கிரந்தி, எண்வகைக்குன்மம், சூலை, பெரும்புண், தொழுநோய், வளிக்குறைவு முதலிய நோய்களை நீக்கும் என்ப.

நற்குணம்

1. குருதியைச் சுத்தி செய்து துர்நீரை நீக்கல்
2. குருதியையும் சுக்கிலத்தையும் பெருக்கல்
3. பசியைத் தூண்டல்
4. கிருமிகளைக் கொன்று புண் புரைகளை ஆற்றல்.
5. உடல் முழுவதையோ அல்லது உள்ளும் புறமுமான உறுப்பின் பகுதியையோ, உறுப்பின் முழுவதையோ பற்றி வியாதிகளைக் குணமாக்கல்
6. முக வசீகரத்தை உண்டு பண்ணல்
7. மறதியை ஒழித்து மூளைக்கு கவன சக்தியைத் தரல்.
8. நரம்புக் கட்டங்களை வன்மையுறச் செய்தல்
9. மனத்தை ஒரு நிலையில் நிறுத்தி ஞானத்தை விருத்தி செய்தல்
10. மூப்பை ஒழித்து ஆயுளை வளர்த்தல்.

தீக்குணம்

இரசத்தைச் சரியான முறையில் முடித்து உண்ணாததால் வரும் குற்றங்கள் அநேகம் என்னும் கருத்தை தேரையர் கூறியுள்ளார்.

மனிதன் செய்யும் பஞ்சமா பாதகங்களை விட, இரசத்தைச் சரியான முறையில் சுத்தி செய்து. பற்ப செந்தூரங்களாய் முடிக்காது கொடப்பது மகா பாதகமாகும். அவ்வாறு உபயோகிக்கும் மருத்துவன் மனிதவர்க்கத்தினுள் சேர்க்கத் தக்கவனல்லன், அவனே கொடி இயமன் ஆவான்.

சுத்தி செய்யாத இரசத்தை உபயோகிப்பதால் உடல் முற்றும் வெந்து போவதன்றிப் பற்களெல்லாம் கழன்று போய்விடும்.

இரச சுத்தா சுத்தகுணம்

சுத்தி செய்யப்பட்ட இரசம், மரணத்தைப் போக்கும் அமிர்தம் போன்றது.

சுத்தி செய்யப்படாத இரசம், மரணத்தை உண்டாக்கும் விடம் போன்றது.

பாதரசத்தின் பத்தியம்

"மற்சமுப்பு சீதோஷ்ண மந்தாதி வத்தெண்ணெய்
துற்சமத்தி யங்கைப்பு தூவுபுளி –யைச்சற்றுங்
கூட்டாமற் சூதங் கொடுப்பருண்டா ரைச்சமன்பாற்

காட்டாமற் றாமிருத்தக் காண்".

இரச சம்பந்தமான மருந்துகள் அருந்துங்காலத்து மீன், உப்பு, மிகுசீதம், மிகு உட்டணம், மந்தப்பொருள், எண்ணெய், மதுபானம் கைப்பு, கார்ப்பு, புளிப்பு சுவையுள்ள பொருள்கள், பெண்போகம் ஆகா.

இரசம் நஞ்சு குறிகுணம்:

குற்றம் உண்டாக்க கூடியது- தூய்மை செய்யாதது, நன்றாக முடிக்கப்பெறாததும், அளவுக்கு அதிகமானதும்.

வாயில் அச்சரத்தைப் போல் புண், பனங்கள்ளைப்போல் வாய் குழம்பும், பற்சந்துகளில் ஈறு கட்டும், வாயில் நீர் பெருகும், தொண்டைவெந்து புண், காது செவிடுபடல், வயிற்றின் மீது பட்டைப்பட்டையாகத் தேமல், போகங்கெடல், கண் பார்வை இழத்தல், செம்படை போல காணல், காலடிகளில் வெடிப்பு. மேலும் பித்தன் போன்று வாய் பிதற்றும், துணிகளை அவிழ்த்து எரியும், வியர்வை பெருகும், தண்ணீரில் முங்கி வெளிவரும் ஆகிய குறிகுணங்களைக் காட்டும்.

இரச நஞ்சு முறிவு:

வெள்ளை முட்சங்கன் இலைச் சாறு அல்லது மிதிப்பாகல் இலைச் சாறு (80 மி.லி) காலையும் மாலையுமாக 3 நாள் கொடுக்கவும்.

லிங்கம்:

பொதுகுணம்

"பேதி சுரஞ் சந்நி பெருவிரண நீரொடுத
காதகடி காசங் கரப்பான்புண் - ணோத
வுருவிலிங்க சங்கதமா யூறுகட்டி யும்போங்
குருவிலிங்க சங்கமத்தைக் கொள்".

"ஆதி யிரதவுருக் காதலாற் சாதிலிங்க
மோதி லிரதகுண முற்றுடலிற் - றீதுபுரி
குட்டங் கிரந்தி கொடுஞ்சூலை வாதமுத
லுட்டங்கு நோய்களையோட் டும்."

பொருள்: தோற்றத்தில் பாதரச உருக்காகிய சிவந்த நிறத்தையுடைய சாதிலிங்கமும், அது சேர்ந்த மற்றைய மருந்துகளும், அந்த இரச குணத்தைக் கொண்டு துன்பத்தை உண்டுபண்ணுகின்ற பேதி, சுரம், சந்நிபாதம், தீராப்புண்கள், அதிமுத்திரம்,காணாக்கடிவிடம், காசம், கரப்பான், சிரங்கு, சொல்வதற்கும் பார்ப்பதற்கும், வெறுப்புத் தோன்றும் பரவு நுணாக்காய்க் கிரந்தி, குட்டம், கிரந்தி, கொடுமை செய்கின்ற சூலை, வாதநோய் முதலியவைகளையும் மற்றும் உடலில் மறைந்து இருக்கும் பிணிகளையும் நீக்கும் என அறிக.

நிலத்தெழுந்த பிணி-பிருதிவி பூத உறுப்புகளில் உண்டாம் நோய்கள் சலப்பிணி – அப்பு பூத உறுப்புகளில் உண்டாம் நோய்கள்.

மற்றும் சரக்குகளுக்கெல்லாம் இலிங்கம் இறையெனவும், மேக நோய்களுக்கு நமன்போன்ற தெனவும் கூறியுள்ளனர்.

அளவு

இதனைப் பத்து உளுந்தெடை (650 மிகி) வரை உள்ளுக்கும், அரை வராகனெடை (2.1 கிராம்) புகை போடுவதற்கும் உபயோகிக்கலாமென்று பைஷஜ கல்பம் கூறுகின்றது.

இலிங்க நஞ்சுக் குறிகுணம்

வாயடி, உண்ணாக்கு, குரள்வளை, பெருங்குடல் முதலியன வெந்து பசும்புண்ணாகிப் பருத்திப்பூவைக் கசக்கி, வெயிலில் இட்டாற்போலிருக்கும். வாயில் காரம் படமுடியாது. உணவு, நீர் முதலியன அருந்துவதற்கும், பேசுவதற்கும், வருத்தத்தைக் கொடுக்கும். வாயில் கெட்ட நாற்றம் வீசி, சுவைகெட்டு, வயிற்றில் எரிச்சலையும் உண்டு பண்ணும். உமிழ்நீர் கெட்டபனங்கள்ளைப் போலவும், காடித் தண்ணீரைப் போலவும் வெண்மையாகவும், குழம்பாகவும் காணப்படும்.

பூரம் (இரசக் கற்பூரம்)

பொதுகுணம்

"இடைவாத சூலை யெரிசூலை குன்மந்
தொடைவாழை வாதமாஞ் சோணி யிடையாதோ
வொக்குரச கர்ப்பூர மொன்றே யளவொடுநல்
இக்குவெல்லத் தேழுநா ளீ."

நல்ல இரச கர்ப்பூரத்தை அளவுடன் கரும்பு வெல்லத்தில் ஏழுநாள் கொடுக்க, இடுப்பைப் பற்றிய சூலை, ஆங்காங்கு எரிச்சலைத் தருகின்ற சூலை, வாத குன்மம், தொடைவாழை, வாதரத்த நோய் முதலியன தீரும்.

மேற்கூறிய செய்யுள் சில நோய்களையே குறிப்பிடினும், பூரத்தை சுரம், மஞ்சட் காமாலை, பித்த தோடம், சீதபேதி, நீர்க்கோவை, விரணசந்தி, ஆறாத விரணங்கள், மேக வியாதி செரியாமை, வாந்தி, பேதி, கிருமிநோய், கீல்வாதம், சொறி, சிரங்கு, மலபந்தம் முதலியவைகட்கும், தலைவலி போன்ற மற்றைய நோய்களுக்கும் உபயோகிப்பதைப் பழக்கத்தில் காணலாம்.

பூர நஞ்சுக் குறிகுணம்

பூரம் விடமித்தால், முகத்தில் செவ்வாப்புப் போல முகப்பரு, வேர்குரு அதிகமாய் உண்டாதல், மார்பின் பள்ளத்தில் பருக்கட்டிப் புண்ணிரணம் காணுதல், இதைப் பக்கத்தில் வலி, வாயில் காரம் படாதபடி புண்ணாதல், பீசவீக்கம், உண்ணாக்கில் விரணம், பேதி, இரத்தக்கழிச்சல் முதலிய துர்க் குணங்களைக் காண்பிக்கும்.

இது விரைவாக நீரில் கரையாது. இது அதிக அளவில் உடம்பிற்குள் சென்றால் நஞ்சுத் தன்மையைக் காட்டும். அவையாவன பல்லீறுகளும், நாவும், வாயும், உந்தியும், தாடையின் உட்பக்கமும் புண்ணாகும். வாய் திறக்க முடியாது. நாவில் நீர் சுரக்கும். அந்நீரில் ஒரு வகையான நாற்றம் வீசும். அதையும் விழுங்க முடியாது.

நஞ்சு முறிவு

"பகருவாய் பூரத்தைப் பாங்குடன்றின் றோர்கட்
கிகலரிய குல்லையினைசாறீக – தகவுடைய
ஏரண்ட நெய்யா மெழிலவுரி வேராகும்
சீர்கொண்ட பாகலிலை தேர்"

1. துளசிச்சாறு (அ) சிற்றாமணக்கு நெய் (அ) பாகல் இலைச்சாறு ஆகியவைகளில் ஒன்றைக் கொடுக்க முறைப்படி 3 (அ) 5 நாள் (அ) நஞ்சு தீரும்வரை கொடுக்கவேண்டும்.
2. அவுரி வேர்ப்பட்டையை வெந்நீர் விட்டரைத்து ஒரு வேளைக்குச் சுண்டைக்காய் அளவு வீதம் காலையிலும் மாலையிலுமாக நஞ்சு தீரும் வரைக் கொடுத்து வர வேண்டும்.
3. நிலப்பனங்கிழங்குக் குடிநீர் : நிலப்பனங்கிழங்கு, வல்லாரை வேர், பொன்னாங்காணி வேர், தண்டுபரங்கி இவற்றை வகைக்கு 10 கிராம் எடுத்து சிதைத்து அதில் 650மிலி நீர் விட்டு எட்டுக்கொன்றாகக் குடிநீர் செய்து காலை, மாலை இரண்டு (அ) மூன்று வாரம் கொடுத்து வர பூரத்தின் வீறு தணியும். (குணபாடம்)

வீரம் (சவ்வீரம்)

பொதுகுணம்

"குன்மமொடு குட்டங் கொடியவனி லத்திரட்டு
துன்மாங் கிசப்பெருக்கஞ் சூலைநோய் - வன்மையுறு
காமியப்புண் ணாதியநோய் கண்டாற்சவ்
வீரனெனுஞ் சாமிநா மத்தையுச் சரி".

சவ்வீரத்தின் நாமத்தை உச்சரித்தாலே குன்மம்,குறைநோய், தீங்கை விளைவிக்கின்ற மகா வாதரோகங்களின் கூட்டம், துர்மாமிச வளர்ச்சி, சூலைநோய்கள், வன்மைபொருந்திய பெண் போகத்தினால் விளைகின்ற (கொறுக்கு, அரையாப்பு, முதலிய) புண்கள் ஆகிய இவை நீங்கும்.

இதனைப் பலவகைப்பட்ட கண்ணோய்களுக்கும் உபயோகிக்கின்றனர்.

சவ்வீரத்தின் அளவு

இதனை 1/32 உளுந்தெடை (2 மி.கிராம்) யிலிருந்து 1/16 உளுந்தெடை (4 மி.கிராம்) வரை உபயோகிக்கலாம். மேற்படின் நஞ்சாம்.

நஞ்சுக்குறிகுணம்

இது நீரில் வேகமாகக் கரையும் தன்மையுடையது. இது உட்கொண்டவுடனேயே குருதியோடு கலந்து விடத்தை மிக வேகமாக விளைவித்துவிடும். இது பிறரைக்கொல்லும் பொருட்டும் தற்கொலை புரிந்து கொள்ளவும் பயன்படுத்துவதுண்டு.

இதை உட்கொண்டால் வாயில் ஒருவகைக் களிம்புச்சுவை உண்டாகும். வாயில் நீருறும். வாய் புண்ணாகி வீங்கும். தொண்டையும் ஆமாசயம் யாவும் வீங்கிப்

புண்ணாகும், வாந்தியும் மலபேதியும், குருதிக் கழிச்சலும் உண்டாகும். எச்சிலைக்கூட விழுங்க முடியாதபடி தொண்டையில் வலி ஏற்படும். முகம் வீங்கிவிடும், உடம்பிலுள்ள தோல் முழுவதும் பட்டை பட்டையாக வெடித்துச் சீலை நீர் வடியும், பக்கம், விலா முதலிய இடங்களில் அதிக நோவும், குடைச்சலுமுண்டாகும், அதிகநீர் வேட்கை, விக்கல், மூர்ச்சை, மயக்கம், வலி முதலியன உண்டாகும். இதோடு சாவும் உண்டாகும்.

நஞ்சு முறிவு

முறையாகச் சவ்வீர மொய்குழலாய் கொண்டால்
சிறுநெருஞ்சிற் சாறுண்ணத் தீரும் - அறையக்கேள்
நீலிவே ராகுமே நெய்ச்சட்டிச் சாறாமே
பாலி தென்னங் கள்ளும் பகர்.

பொருள்

சிறு நெருஞ்சிற்சாறு, நீலிவேர்ப்பட்டைக் கல்கம், நெய்ச்சட்டிக் கீரைச்சாறு, தென்னங்கள் இவைகளிலொன்றை நஞ்சின்வன்மைக்குத் தக்கஅளவில் நஞ்சு முரியுமட்டும் கொடுக்க வேண்டுமென்பதாம்.

கோழிமுட்டை வெண்கருவைத் தண்ணீர் அல்லது பாலுடன் கலந்து அடிக்கடி கொடுத்து வந்தாலும், இளநீர், அருந்தினாலும் வீரத்தின் நஞ்சு நீங்குமென்ப. இதனை,

“அண்டத்தின் வெண்கருவை யாவின்பா லிற்கலந்
துண்டுவர வீரனு னுரமகலுங் - கண்டரிவாய்
ஏணற்கொடியே யிளநீ ரருந்திடினு
மாணப்பெருமை வழுத்து.

என்ற கருவூரார் தண்டகச் செய்யுளால் உணர்க.

Plants are the best natural source of medicine the world. According to Food and Agriculture Organisation (FAO) reported that on an average 70-80% of people in the world encroaches herbal medicines to prevent and cure diseases. And it is estimated that about 25% of synthesized drugs are from medicinal plants.

Tribulus terrestris:

Pharmacological actions:

“நல்ல நெருஞ்சிலது நாளுங்கி ரிச்சாரத்தை
வல்ல சுரமனலை மாற்றுங்காண்-மெல்லியலே!
மாநிலத்தில் கல்லடைப்பும் வாங்காத நீர்கட்டும்
கூனுறுமெய் வாதமும் போக்கும்.
மேகவெட்டை நீர்சுறுக்கு வீறுதிரி தோடம்புண்
வேகாசுர தாகவெப்பம் விட்டொழியும்-போகந்
தருஞ்சின மதலைமொழித் தையலே! நல்ல
நெருஞ்சி லதனை நினை.”

Nourishing, its medicinal property pacifies vata dosha, increases BMR, relieves spasmodic pain and cures bladder ailments such as oliguria, burning micturition, anuria and leucorrhoea.

Recent studies endorsed that it has specific activities in improving sexual function(aphrodisiac), cardiac protection and also has anti-urolithic, anti-diabetic, anti-inflammatory, anti-tumour, anti-spasmodic and anti-oxidant activities to enhance health. In Tribulus terrestris, there are 108 kinds of steroidal saponins were identified in experiments. The major phytoconstituents responsible for the medicinal uses are kaempherol- glucoside, Terrestrin (C, D, E) - saponin, Tribulusterine and vanillin-Alkaloids and Furostanol- glycoside (46).

In experience, the ash form of whole plant is claimed to be effective in treating Rheumatoid arthritis. The whole plant used in the treatment of male infertility and induces spermatogenesis.By highlighting the anti-oxidant property, a research reported that it reduces the oxidative stress and free radicals formation induced by mercuric chloride treatment.(47)(48)

Moringa oleifera:

Pharmacological actions:

“விழிகுளிரும் பித்தம்போம் வீறருசி யேகும்
அழிவிந் துவும்புஷ்டி யாகும்-எழிலார்
ஒருங்கையக லாககற் புடைவா ணகையே
முருங்கையின் பூவை மொழி.”

The pharmacological activities studied in Moringa oleifera were antiproliferation, hepatoprotective, anti-inflammatory, anti-nociceptive, anti-atherosclerotic, oxidative DNA damage protective, anti-peroxidative and cardioprotective.

Especially siddha literature describes that M.oleifera flower can be used as emmenagogue and it also induces spermatogenesis with increased secretion of semen from seminal vesicle.

Several studies reported that it is effective in treating infections, enlarged spleen, neuronal disorders, fever, cough, ulcers, dyspepsia and dysmenorrhoea. The major phytochemical pterygospermine is the antibiotic principle against gram positive, negative and acid fast bacteria.(49) Recent researchshows that it can reduces peroxidative reaction in the testicular membrane and increases healthy spermatozoa

production.(50) It is reported that M.oleifera reduces testicular toxicity induced by mercuric chloride.(40)(48)

Mother's milk:

Human breast milk is uniquely superior for infant feeding in the period of 'crucial window' age. Majorly it contains an average of 1.1% protein, 4.2% fat, 7.0% carbohydrate and 72 kcal of energy per 100g of milk.(51) It is documented that feeding milk decreases infectious diseases (bacterial meningitis, bacteraemia, diarrhoea, respiratory tract infection, otitis media and sepsis). It contains vitamin C and E, superoxide dismutase, catalase and glutathione peroxidase and thus proves that it has antioxidant properties.(52) Apart from this, it enhances the immune mediated mechanisms and maintains the texture of tissues in human. Antioxidants are considered to be the supreme power to avoid cell damage and DNA damage. A protein lactoferrin (iron-binding transferrin protein) which is abundantly present in breast milkbinds to iron which attenuates the conversion of free radicals.Abundant antioxidants were present in human breast milk. But the antioxidants level decline day by day, if it is refrigerated. Another study reported that there is a marked increase of an oxidative stress marker malondialdehyde in refrigerated milk and not in frozen milk samples. But there is a decreased glutathione peroxidase activity in both refrigerated and frozen milk samples.

தன்னியமென் றோதிச் சருவதோ ஷங்கள்போம்
உன்னிய தாப மொழியுங்காண்-சந்நியொடு
வாதசுரம் பித்தசுரம் வன்கபச்சுரந்தனியுங்
கோதில்வன் மையுண்டாங் கூறு.

Honey:

Honey is the worldwide recognised nutritive sweetest liquid for human wellbeing. Since ancient Egyptians, Greeks, Romans and Chinese has been used traditionally used in medicinal field to treat gastric ulcers, sore throat, cough and ear aches. (53) On external application, the therapeutic effect heals the wound fast, cleans and clears the infectious tissues and reduces inflammation. Naturally it composed of 82.4% carbohydrates, 38.5% fructose, 0.5% protein, 31% glucose and 17.1% water.

In Siddha system of medicine, it can be used as demulcent, laxative, antiseptic agent, expectorant, astringent, best appetizer, general tonic and hypnotic. Especially in

children it act as diuretic and relieves indigestion. Not only as internal adjuvant, plays a potential role in healing wounds externally. Though this this system elaborates the purification method of honey for its adjuvant use in the treatment period. In ancient period, Egyptians and Asians used honey to delay the action of decomposition in dead bodies. The same events evidenced among Greek population. Eventually in this system, it plays a vital role as adjuvant in enhancing the efficacy of the therapeutic dose of medicine. Some of the literatures documented the importance of honey as adjuvant are given below:

“அனுபானத் தாலே யவிழ்தம் பலிக்கும்
இனிதான சுக்கு ன்னலிஞ்சி-பினுமுதகங்
கோமயம் பால்முலைப்பால் கோநெய்தேன் வெற்றிலைநீர்
ஆமிதையா ராய்ந்துசெய லாம்.”

“அனுபான மாய்ப்பின் அவிழ்தமுமாய்த் தோன்றி
கனமான தேகநிலை காட்டிப்-பினுமே
யரசன் முதல்வோ ரையுமாட்டு வித்தாலே
பிரசத் தினாற்போம்.”

தேரன் பொருள்பண்பு நூல்.

3 Preparation and Standardisation

Background:

Preparation and standardization always play a trivial role in pharmacological understanding of any traditional drug formulation. This becomes more complicated if the final drug authentication procedures of the particular drug formulation are not very well known. Few Siddha scholars of present time have attempted to establish standards for commonly used Siddha formulations, such as *Amukkarā cūrṇam* (54) *Kānta centūram*(55) *Liṅka centūram* No. 1(56) Psoriatic Care 777 oil (57), *Mattaṉ tailam*(58) and. *Tāḷakakaruppu*(59) etc. However, most of the works fail to address the exact need for standardization. Several *Pāṭāṇam* (arsenic based formulations) of Siddha system are currently researched for its clinical efficacy in Malignancies.(60)(61)(62)(63). In those works, the standard range has been fixed by preparing the formulation at least for three times. As far as the herbo-metallic siddha drugs (HMPs) are concerned, traditional authentication procedures are well established only for *paṟpam* and *centūram*. Though no general authentication procedures are known for accessing the quality of *meḻuku* based HMPs in classical Siddha texts, few vaittiyarkaḷ in Kanniyakumari district assert the completion of *meḻuku* type of HMPs by rubbing them in skin and placing them in direct sunlight. If any glittering particles are seen or if any skin irritation is observed, the drug is deemed to be incomplete and the entire process has to be repeated again. Absence of the above mentioned signs ascertain that the drug preparation is complete. As our trial drug *Panca Cuta Meluku* (PCM), has 5 inorganic and three organic ingredients, the preparation and standardization should be performed with at most care to avoid any errors. This chapter primarily deals with the preparation process and standardization process espoused in present work.

Materials and Methods:

The preparation of any HMPs consists of following standard procedures:

1. Collection of raw materials
2. Authentication of the raw materials
3. Purification of raw materials
4. Preparation of drug

5. Final authentication of drug

The same process was followed in this work.

Collection of Raw Materials:

The raw drugs *Iracam, Liṅkam, Pūram, Vīram* and *Iraca centūram* were procured from local store in Nagercoil, Kaniyakumari District. The Mercury (Batch no: 089805) was purchased from Spectrum Reagents and Chemicals pvt.ltd.*Neruñcil camūlam*and Muruṅkaipū were collected from regions around Tuckerammalpuram, Tirunelveli. Mother's milk were generously donated by lactating mother's hospitalized in various private hospitals in and around Palayamkottai, Tirunelveli, Tamil Nadu, India. Honey was purchased from outlet of Khadhi Kraft, Gandhigram Lakshmi SevaSangam (LSS).

Authentication of raw materials

The raw materials*Iracam, Liṅkam, Pūram, Vīram* and *Iraca centūram* were authenticated by experts at Department of Chemistry, Central Council for Research in Siddha, Chennai. *Tribulus terrestris* (*Neruñcil camūlam*) [Voucher Specimen No: T170619006T] & *Moringa oleifera* (*Muruṅkaipū*) [Voucher Specimen No: M170619005O] were authenticated by experts at Siddha Medicinal Plants Unit, (Under CCRS), Mettur, Tamilnadu.

Purification

The purification protocol was performed as per the descriptions in classical Siddha text.(25)

- *Iracam* was purified by filtering in a white cloth for 1000 times.
- *Liṅkam* and *Iraca centūram* were purified by soaking in lemon juice and mother's milk for a period of one day each.
- A mixture of black betel leaves and black pepper (10 grams each) ground with sufficient quantity of water and dissolved in 2L of water was placed insidean earthen pot. *Pūram*was tied in a cotton cloth and was suspended inside an earthen pot by tying on a stick placed on neck of the earthen pot in such a way that it gets immersed inside the liquid. Then the earthen pot was heated till the

liquid in pot was reduced to one-fourth. The left over material was washed and dried.

Figure 3. 1: Image of traditional set up of purification process of *Pūram*

- A mixture of 10 grams of camphor dissolved in 2 L of tender coconut was placed inside the earthen pot and*vīram*was tied in a cotton cloth and was suspended inside the earthen pot by tying on a stick placed on neck of the earthen pot in such a way that *vīram* doesn't get immersed inside the liquid.
- *Tribulus terrestris* was purified by removing the dried, pale leaves and washed in running water.
- *Moringaoleifera* was purified by removing the stalks, dried flowers, polygons and qurcones and washed in running water.
- Mother's milk was purified by heating in a silver vessel followed by filtration.

Figure 3. 2: Image of traditional set up of purification process of *vīram*

Preparation of medicine:

The protocol espoused in preparation of PCM is described in Figure.3.3. The PCM is prepared by traditional Siddha method call as *curukku kuṭuttal*. Briefly, all the metallic ingredients were ground into fine powder as per the order mentioned in Figure.3.3followed by heat treatment in an earthen pan placed over a stove. Afluid containing equal mixtures of juices extracted from *Moringa oleifera*, *Tribulus terrestris* and mother's milk is added in drops in such a way that the sample in earthen pan doesn't become dry. The temperature is maintained between 250-300^{o}C (*kamalākkiṉi*).The process is continued till all the juices are utilized.

Note: *The whole preparatory process should be performed under the shade of a Tamarind tree. The persons engaged in preparation should consume only milk and rice during the day of preparation. Head cap, face mask and gloves should be worn all through the process.*

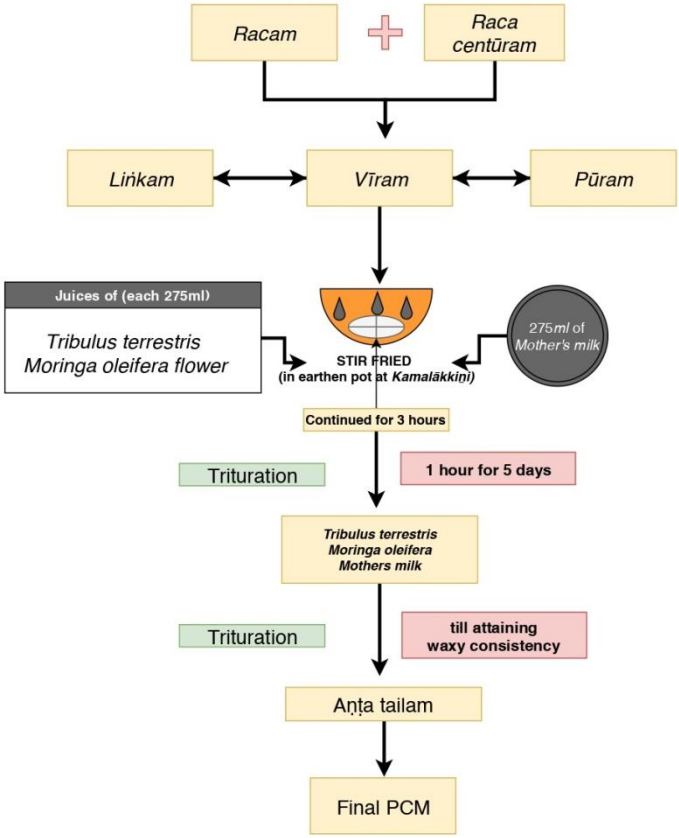

Figure 3. 3: Schematic representation of preparation protocol of PCM

PLIM Parameters:

Various analytical specifications were performed as per the Pharmacopoeial laboratory of Indian Medicine guidelines and the reference followed for analysis is tabulated below.

Table 3. 1: Methods followed for PLIM parameters analysis

S No	Parameters	Reference of test methods
1	Appearance	IP Vol-I, 1996, p7
2	Total solids	IP Vol-I, 2014, p277
3	Total Ash	IP Vol-I, 2014, p98
4	Acid insoluble ash	IP Vol-I, 2014, p98
5	Loss on Drying at 105°C	IP Vol-I, 2014, p162
6	Carbohydrates	Biochemical Methods, Sadasivam.S,2005,p8-9

Qualitative analysis:

Presences of various contents in PCM are determined using following methods.

Test for calcium - 2ml of the above prepared extract is taken in a clean test tube. To this add 2ml of 4% ammonium oxalate solution.

Test for sulphate - 2ml of the extract is added to 5% barium chloride solution.

Test for chloride - The extract is treated with silver nitrate solution.

Test for carbonate - The substance is treated with concentrated HCl.

Test for starch - The extract is added with weak iodine solution.

Test for ferric iron - The extract is acidified with glacial acetic acid and potassium ferro cyanide.

Test for ferrous iron - The extract is treated with concentrated nitric acid and ammonium thiocyanate solution.

Test for phosphate - The extract is treated with ammonium molybdate and concentrated nitric acid.

Test for albumin - The extract is treated with esbach's reagent.

Test for tannic acid - This extract is treated with ferric chloride.

Test for unsaturation - Potassium permanganate solution is added to the extract.

Test for the reducing sugar - 5ml of benedict's qualitative solution is taken in a test tube and allowed to boil for 2 minutes and add 8-10 drops of the extract and again boil it for 2 minutes.

Test for amino acid - One or two drops of the extract is placed on a filter paper and dried well. After drying 1% ninhydrin is sprayed over the same and dried it well.

Test for zinc - The extract is treated with potassium ferro cyanide.

Characterisation:

TGA (Thermo-gravimetric analysis) was performed by placing 2-5 mg of sample in an alumina cup and heated at the rate of 10°C/min up to 1000°C in a nitrogen atmosphere with a flow rate of 100 ml/min using an SDT Q600 (TA Instruments, USA).

FTIR (Fourier transform infra-red) spectra of samples were recorded between 4000 and 400 cm−1 in the IR spectrometer (Spectrum 100, Perkin Elmer, USA) using KBr pellet method.

The elemental composition of the samples was determined using XRF (X-ray fluorescence) spectrometer (S8 Tiger, Bruker AXS, Germany) using a 4 kW Rhodium anode X-ray tube by using boric acid pellet method.

The crystallinity of the samples were analyzed using an X-Ray diffractometer (D8 Focus, Bruker, Germany), by irradiating with Cu-kα radiation. The analysis was performed from 10ºto 60 º(2θ) with a step size of 0.01 º.

Note: *As the samples of PCM-final was in waxy consistency, 25 g of PCM-FINAL is heated for 15 minutes at temperature between 250-300ºC and the remaining left over sample (2.78g) is given for XRD analysis. PCM-BT was given as such for XRD analysis.*

Results:

The difference in weight of the raw material before and after purificationis shown in table 3.2.

Table 3. 2: Difference in weight after purification of raw materials

Drug	Weight (g)		Observations
	Before purification	After purification	
Iracam	100	95	At the end of each time, it is noted that there is a presence of blackish powder
Pūram	100	80.9	No notable changes were observed
Vīram	100	95.3	No notable changes were observed
Liṅkam	100	98.7	No notable changes were observed
Iracacentūram	100	97.5	No notable changes were observed

Preparation:

50gof *Iracam, Liṅkam, Pūram, Vīram* and *Iraca centūram* are subjected to heat with 275 ml of *Neruñcil camūlam* juice,*Muruṅkaipū* juice and *Tāypāl* and 280g of final drug was obtained.

Standardization:

Primary chemical analysis of PCM showed the presence of calcium, sulphate, chloride, ferrous form of iron, unsaturated compounds, reducing sugar and amino acid. Few substances such as carbonate, starch, ferric form of iron, phosphate, albumin, tannic acid and zincare absent in PCM. The results of PLIM standards is shown in table 3.3

Table 3. 3: Results of PLIM standards for PCM

S No	Parameters	Results
1	Appearance	Black coloured wax

2	Total solids	10.68%w/w
3	Total Ash	5.678%w/w
4	Acid insoluble ash	0.9728%w/w
5	Loss on Drying at 105°C	4.770%w/w
6	Carbohydrates	4.825.%w/w

TGA analysis:

The two different methods (rise of temperature at 10°C / min and 20°C/ min) of TGA analysis of PCM is shown in figure 3.4. This shows that maximum degradation of the substances in PCM happen around 450°C.

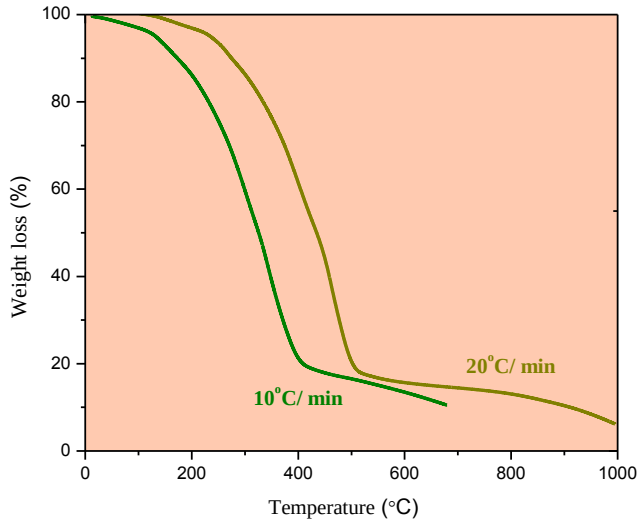

Figure 3. 4: TGA analysis of PCM

IR spectroscopy:

IR spectra of PCM-BT, PCM – Final and AT is shown in figure. 3.5. Peaks at 474 cm^{-1} and 1614 cm^{-1} in IR spectra of PCM-BT were assigned to the stretching vibrations of $HgCl_2$ and Si-C_4linear vibrations respectively. The peaks at 3526 cm^{-1} and 3585cm^{-1} were due to the OH stretch. Peaks at 1629 cm^{-1}, 2896 cm^{-1} and 2855 cm^{-1} in AT shows the presence of symmetrical carbonyl stretching and bending. Peaks at 1038 and 1078 could be due to in plane bending of C-H bond. The peaks at 3007 and 3389 show the OH vibrations.

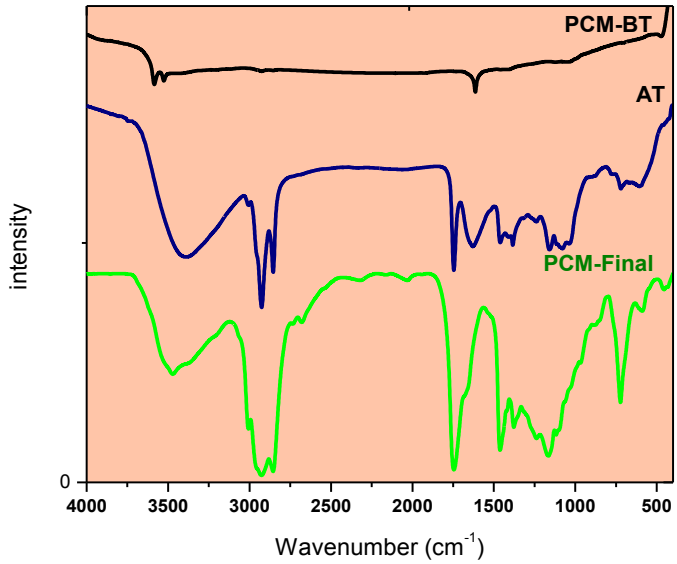

Figure 3. 5: IR spectra of PCM- Final, AT and PCM-BT

The peaks at 1746 cm^{-1}, 2048 cm^{-1}, 2061cm^{-1} and 2129 cm^{-1} could plausibly due to the overtones produced by vibrations of carbonyl bonds. PCM-Final showed peaks corresponding to Hg-S stretch of Cinnabar at 589 cm^{-1} and 1121 cm^{-1}(64). Peaks corresponding to Hg-Cl stretch of $HgCl_2$ is noted at 464 cm^{-1} and 723 cm^{-1}. A shift of 9 cm and 6 cm is observed in 1168 cm^{-1} and 1378 cm^{-1} peaks.

X-Ray Fluorescence spectroscopy (XRF):

Table 3. 4: Elemental composition and oxidation state of PCM analysed using XRF

Oxide form of PCM-BT		Elemental form of PCM-FINAL		Oxide Form of PCM-FINAL	
Formula	**Concentration (%)**	**Formula**	**Concentration (%)**	**Formula**	**Concentration (%)**
Hg	87.5	**Hg**	39.2	**SO_3**	31.6
PbO	2.5	**S**	17.4	**Hg**	27.0
Cl	1.3	**Cl**	14.3	**Cl**	10.3
SiO_2	0.6	**K**	10.3	**K_2O**	9.1
MnO	0.2	**Ca**	6.0	**SiO_2**	6.0
Others	7.9	**Si**	1.7	**CaO**	5.9
		P	1.7	**P_2O_5**	2.5
		Mg	1.6	**MgO**	2.3
		Others	7.8	**Others**	5.2

The elemental composition of PCM-BT and PCM-FINAL analysed using XRF is tabulated in table.3.4.

X-Ray Diffraction spectroscopy:

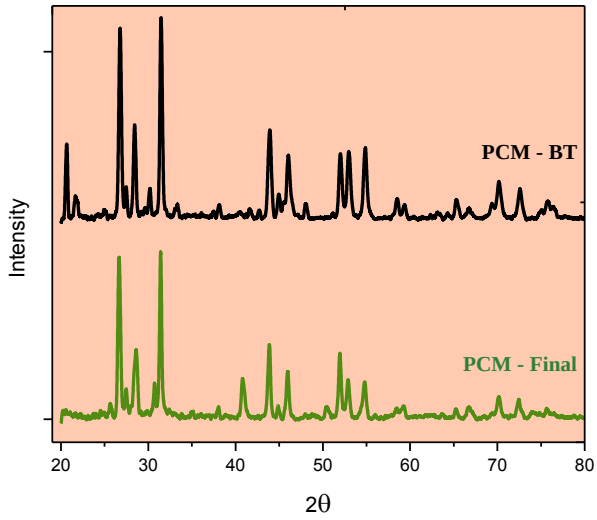

Figure 3. 6: XRD spectra of PCM-BT and PCM-final

XRD spectra of PCM-BT, PCM-Final and AT are shown in Figure 3.6. PCM-BT shows significant peaks at 31.46 **2θ**, 26.77 **2θ**, 28.43 **2θ**, 43.92 **2θ**, 20.64 **2θ**, 54.86 **2θ**, 52.98 **2θ**, 52.01 **2θ** and 46.04 **2θ**. Among this, peaks at 20.649 **2θ**, 21.647 **2θ**, 28.438 **2θ** and 30.118 **2θ** were assigned to the diffraction of Hg-Clbonds in $HgCl_2$(65). Peaks at 28.43 **2θ**, 21.67 **2θ**, 33.32 **2θ**, 43.92 **2θ** and 46.24 **2θ** were assigned to Hg_2Cl_2(66). Peaks at 26.77 **2θ**, 31.46 **2θ**, 43.92 **2θ** and 46.04 **2θ** were assigned to HgS vibrations(64). Peaks at 52.015 **2θ**, 52.98 **2θ** and 54.86 **2θ** could not be assigned to any of the compounds but it represents the crystalline nature of the sample. PCM-Final shows significant peaks at 31.41 **2θ**, 26.65 **2θ**, 43.87 **2θ**, 28.6 **2θ**, 51.94 **2θ**, 45.97 **2θ**, 40.81 **2θ**, 52.89 **2θ**, 54.79 **2θ** and 30.73 **2θ**. Among this, peaks at 31.41 **2θ**, 26.65 **2θ**, 43.87 **2θ** and 45.97 **2θ** were assigned to the HgS diffraction(64). Peaks at 52.89 **2θ** and 54.79 **2θ** could not be assigned to any of the compounds but it represents the high crystalline nature of the sample.

Discussion:

The major perspective of this work is to prepare and characterise the PCM by employing contemporary methods without losing the traditional essence. After getting authentication of raw materials fromChemistry and Plants unit department under Central Council for Research in Siddha, the drugs were purified as per literature and PCM was successfully prepared. Following preparation, thePharmacopoeial standards

were fixed as per the PLIM guidelines. Preliminary qualitative analysis of PCM-final showed the presence of presence of calcium, sulphate, chloride, ferrous form of iron, unsaturated compounds, reducing sugar and amino acid and absence of carbonate, starch, ferric form of iron, phosphate, albumin, tannic acid and zinc. As the IR spectra of PCM-final showed the presence of hydrocarbon peaks, the presence of organic substances is confirmed. Hence, TGA was performed to evaluate the amount of organic and inorganic substances present in PCM-final. But quantity of organic substances could not be evaluated as most of the salts of mercury has melting point less than 400^{o}C (67)(68)(69)(70)(71)and the weight loss was approximately 95% at 1000^{o}C. This was quite surprising as the PCM-BT was heated at *kamalagni* (approximately 250-350^{o}C), and we could see an average of 50% decrease in mercury during the preparation that lasted for more than 3 hours. However, the weight loss during TGA analysis at 400^{o}Cwas 5-10% depending on the rate of rise in temperature. This implies that the mixture of juices and mother's milk would have played a crucial role in prevention of evaporation of mercury. Albeit, this can be confirmed only after performing various control experiments. As the percentage of organic could not be established, XRF was performed to understand the elemental nature of PCM-Final. The presence of significant quantity of K, Ca, PCM-BT apart from Hg and Pb that was present before *curukkukuṭuttal* implies that K and Ca would have either come from the mixture of juices along with mother's milk or from *Aṇṭatailam*. Moreover, shift of hydrocarbon peaks to 6-9 cm^{-1} in IR data denotes the presence of organometallic complex in PCM – final.

Conclusion:

From the above discussion following conclusions were drawn.

1. The PCM was successfully prepared as per the descriptions in the Siddha classics. The standardization parameters were established following the PLIM guidelines.
2. Preliminary qualitative analysis showed the presence of calcium, sulfate, chloride, ferrous form of iron, unsaturated compounds, reducing sugar and amino acid and absence of carbonate, starch, ferric form of iron, phosphate, albumin, tannic acid and zinc
3. There is no organic content in the PCM in the before trituration.

4. Mercury, sulfur, chlorine, potassium, calcium, silicon, phosphorus and magnesium were the major elements found in the final PCM.
5. The sample before trituration predominantly contains HgS and HgCl2.
6. The final PCM was found to contain notable quantity of organic materials in it along with significant quantity of HgS. The presence of HgCl2/Hg2Cl2 is very limited in final PCM.

4 Pre-Clinical Experiments

In this expeditious century, the drug resistance was the common trend in medical system which leads to the innovation of new drugs. In the development of new drugs toxicological screening is essential before human consumption. The US Food and Drug Administration states that for the development of drug, it is necessary to screen the pharmacological activity and toxicity study in animals. These type of animal toxicity studies began in 1920 still in research. In order to evaluate the safety profile of a drug toxicity studies were carried. Many literature formulations in Siddha system of medicine hold much medicinal value but it is vital to record the safety profile of the individual drug. Here the medicine PCM already exists with toxic symptoms and their specific antidotes along with general properties. Thus it was concerned to establish the toxicological profile by animal experiments as per OECD guidelines.

Materials and Methods:

Adult Wistar rats (6-8 weeks) of both sexes procured from the Central Animal Facility, SASTRA University were used for this study. The animals were kept at 21±2 °C with a 12 h light/dark cycle, with free access to standard rat pellet diet and water ad libitum. The experimental protocols were performed after obtaining the necessary approval (Approval number: CAF/195/S/IAEC/RPP) from the Institutional Animal Ethical Committee (IAEC) of SASTRA University.

Study Plan - Acute Oral Toxicity Study:

This study was performed in compliance with OECD 425 (Up and down method) guidelines(72). The main test consists of a single ordered dose progression in which animals are dosed, one at a time, at a minimum of 24-hour intervals. The first animal receives a dose a of 175mg/kg and observed at least once during the first 30 minutes after dosing, periodically during the first 24 hours (with special attention given during the first 4 hours), and daily thereafter, for a total of 14 days, except where they need to be removed from the study and humanely sacrificed for animal welfare reasons or are found dead.. If the animal survives for 24 hours, the dose for the next animal is increased by [a factor of] 3 .2 times the original dose; if it dies, the dose for the next

animal is decreased by a similar dose progression. (Note: 3.2 is the default factor corresponding to a dose progression of one half log unit). Each animal should be observed carefully for up to 24 hours before making a decision on whether and how much to dose the next animal. That decision is based on the 24-hour survival pattern of all the animals up to that time.Dosing continues depending on the fixed-time interval (e.g., 24-hour) outcomes of all the animals up to that time. The testing stops when one of the following stopping criteria first is met:

(a) 3 consecutive animals survive at the upper bound;

(b) 5 reversals occur in any 6 consecutive animals tested;

(c) At least 4 animals have followed the first reversal and the specified likelihood-ratios exceed the critical value.

Body weight was measured once in a week and feed consumption was measured daily. Animals were sacrificed at day 14 and gross pathology, if any were noted and taken for further investigation.

Study Plan -28-days repeated oral toxicity

This study was performed in compliance with OECD 407 guidelines(73)and dose administration was performed as per the descriptions in table 1.1.Animals were totally divided into 7 groups (Normal control, vehicle control, low dose PCM treated, medium dose PCM treated, high dose PCM treated, Satellite control satellitehigh dose PCM treated) with 10 animals (5male and 5female) in each group. Normal control and Satellite control animals were administered only with distilled water. Vehicle control animals were administered with vehicle (honey) without any drug. Low, moderate and high drug treated groups were administered with different concentrations of PCM dispersed in vehicle (honey). The drug/vehicle was administered for a period of 28 days orally using oral gavage tube. All the animals were sacrificed after 28 days and required biological samples were collected for further analysis. Body weight changes were noted in alternate days and feed consumption was noted daily.

Table 4. 1: Study plan: 28-days repeated oral toxicity study

Animal Group	No. of animals (male +female)	Dose administration
Control	5+5	Distilled water
Vehicle control	5+5	Honey
Low dose	5+5	Therapeutic dose
Medium dose	5+5	5 x Low dose
High dose	5+5	10 x Low dose
Satellite control*	5+5	Distilled water
Satellite high dose*	5+5	10 xLow dose

Dose:

As the human therapeutic dose of PCM is already established in the Siddha texts (100mg/dose for normal adult with 2-3 times a day) (4), considering the weight of a normal adult as 60kg, animal equivalent dose was calculated using allometric dose translations(74).

Animal equivalent dose (rat)=Humandose×HumanKm/AnimalKm

Animal equivalent dose (rat)= 1.538 ×37/6= 9.48mg/day~ 10mg/day

Considering the drug was administered twice a day, AED is 20mg/ day.

Animal equivalent therapeutic dose was fixed as low dose. Medium and high dose were fixed as 5 times and 10 times that of the low dose (therapeutic dose) respectively.

Cage side Observations:

The toxicity signs from activity, Mobility, nature of the Pupil, Piloerection, Tremor, Splenomegaly, Diarrhoea, body temperature, and pallor were noted and scored accordingly. Overall summation of all the cumulative score was calculated and the observed toxicity status was graphed. In Sub acute study period, individual animals were observed on every 7th day for any abnormalities and most of the notable changes were observed from day1 to day 14. Those changes were assumed with the following scorings and cumulative scores were done.

1. Activity:

The activity refers to the behaviour of the animal such as restlessness, jumping, sluggishness, paw lifting, aggressiveness, and alertness were considered to be abnormal and scored as ‘1’, Whereas normal behaviour were scored as ‘0’.

2. Mobility:

The mobility mean to be whether the animal moves freely (Score = 0) or moves by any external stimuli (Score = 1) or immobile (Score = 2) were noted.

3. The nature of Pupil:

To assess the state of pupil in moribund animals, scoring were done in order; normal- '1', dilated- '2' and constricted- '3'.

4. Piloerection:

Piloerection observed over the hairs of the body. The absence of piloerection were scored as '0' and presence of piloerection is scored as '1'.

5. Tremor:

Involuntary shaking movement or involuntary muscle contraction and relaxation were recorded. Intentional tremor is an important sign seen in animals which are intent for moving. Absence of tremor is scored as '0', Tremor occurs during intention for walking is considered as mild tremor which is scored as '1', tremor during movements(kinetic movement) is noted a moderate tremor represented as score '2', and severe tremor (persistent tremor) as'3'.

6. Internal organs- palpation:

Internal organs were palpated regularly once in 7 days and no palpable changes were noted except spleen. The changes of spleen was almost seen on 8^{th} days and recovered back within 3 days. The changes were scored with the following:

Normal spleen (no marked difference) = '0'

Mild splenomegaly (elevation during respiration) = '1'

Moderate splenomegaly (mild elevation) = '2'

Severe splenomegaly (prominentelevation)= '3'

7. Diarrhoea:

The condition of diarrhoea was accessed based on its faecal consistency and shape of fecal matter present in the cage on the next day morning. The faecal matter in definite shape is scored as '0' and ill definite shape (spread in nature) is scored as '1'.

8. Body temperature:

Temperature of the individual animal were monitored at the time of moribund. Normal body temperature is scored as '0', hypothermia is scored as '1', and hyperthermia isscored as '2'

9. Pallor:

The paleness of the skin especially in tail, ear lobes and paws of animals were observed. Absence of paleness is scored '0' and presence of Paleness scored as '1'.

Overall toxicity assessment and scoring system

The toxicity signs from activity, Mobility, nature of Pupil, Piloerection, Tremor, Splenomegaly, Diarrhoea, body temperature, and pallor were noted and scored accordingly. Overall summation of all the cumulative score was calculated and the observed toxicity status was graphed.

Haematology and Biochemical Analysis:

About 1mL of blood samples were collectedfrom all the animals on 29th day from retro-orbital plexus before sacrifice.Haematology analysis was performed in whole blood using hemocytometer.

The serum was separated by allowing the remaining blood sample to coagulate at room temperature followed by centrifugation at 605rcf for 10 minutes. Biochemical analysis was performed in this serum using following methods

Histopathology Analysis:

After sacrifice, the specific organs were isolated, washed with cold saline, weighed and finally fixed in 10% buffered formalin solution for histopathological studies. The fixed tissues were embedded in paraffin and the sections were cut in 3-5µm slices and were stained using haematoxylin and eosin. The stained tissues were observed under light microscope. The scoring was given to the pathological features seen in the slide as 0 for normal, 1 for minimal, 2 for mild, 3 for moderate, and 4 for marked and 5 for severe.

Isolation of protein and Western blotting:

Total protein from the collected rat tissues were isolated using 10X cell lysis buffer (V8571,Promega, USA) containing protease inhibitor cocktail purchased from Sigma, USA (P8340). Briefly, to the cell pellet from 34.8 mm petri dish 30 µL of lysis buffer was added. The cell mixture was pipetted out persistently for 30 sec followed by centrifuging at 12000 rpm at 4ºC for 5 min. This step was repeated 3 times followed by centrifugation at 14000 rpm at 4ºC for 20 min. The resulting supernatant is then stored in a fresh vial until further use.

For western blotting the following steps were followed:

1. 50µg of protein separated earlier was utilized. This protein lysate was loaded onto a 10% sodium dodecyl sulfate (SDS) gel and was ran at 110Voltage (V).
2. After electrophoresis the protein ran in SDS gel was transferred to an immune-blot polyvinylidene-di-fluoride (PVDF) membrane (1620112, Bio-Rad) at 100V for 1 hour using Trans-Blot Turbo Transfer System (Bio-Rad).
3. After transfer of protein the blot was incubated for 1 hour with 5% BSA prepared in 1X tris buffer saline-tween 20 (TBST) buffer on a gel rocker for blocking the surface from cross liking of antibody with the membrane.
4. After blocking, the blot was incubated overnight at 4ºC with respective primary antibody. The primary antibody was prepared in 5% bovine serum albumin in 1X tris buffer saline (TBS) buffer.
5. The next morning the blot was washed thrice with TBS before adding HRP conjugated secondary antibody specific for the primary antibody. The blot was then incubated at room temperature for 2 hour in a shaker.
6. The blot was again washed thrice with TBS before adding substrate (#1705060-Clarity Western ECL Substrate, Bio-rad).
7. Chemiluminescence signals from the immune blot membrane were detected using Chemi Doc XRS system, Bio-Rad after adding the substrate.

The images were captured using Quantity one 1-D analysis software provided by Bio-Rad and the band intensity was then analyzed using Image J software. The following antibodies were used for the detection of ICAM-1 and VCAM-1. The protein levels were normalized to GAPDH protein levels in all the experiments.

a. Anti- ICAM-1 rabbit polyclonal antibody (4915S, Cell signaling technology),

b. Anti-VCAM-1 rabbitmonoclonal antibody (13662S, Cell signaling technology)
c. Monoclonal GAPDH antibody (Santa Cruz, USA).

Statistical Analysis:

All the results are expressed as mean ± SD (n= 8-10). One way ANOVA, followed by Dunnett's post hoc test was performed to show the significance of the test results. $p<0.05$ was considered to be statistically significant.

Results:

Acute Oral Toxicity Study

Cage side observations:

As first animal administered with 175mg/kg of PCM survived for 24 hours, second animal was administered with 550mg/kg on next day. After 24, as no mortality were noted, third animal was administered with 2000mg/kg on third day. Other two animals were administered 2000mg/kg on fourth day and fifth day respectively as the third animal survived for 48 hours.However, the third animal administered with of 2000 mg/kg dose died 4 days after PCM administration with decline in feed consumption (0-2%) from 3rd day.

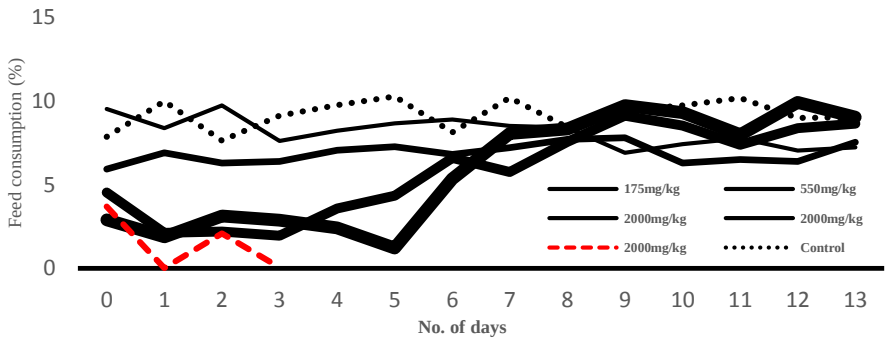

Figure 4. 1: Acute toxicity study: Feed weight with respect to body weight

Feed consumption with respect to body weight of male animals are expressed in percentage and is shown in Figure 4.1. Average feed consumption of control and 175 mg/kg is about 8-10 %. 550 mg/kg drug administered animal consumes an average of 6-8%. 2000 mg/kg drug administered animal consumption declines initially up to day 5 with an average of 5-1% and increases to 7-10%. An animal with 2000 mg/kg dose died on day 3 with minimum feed consumption of 0-2%.

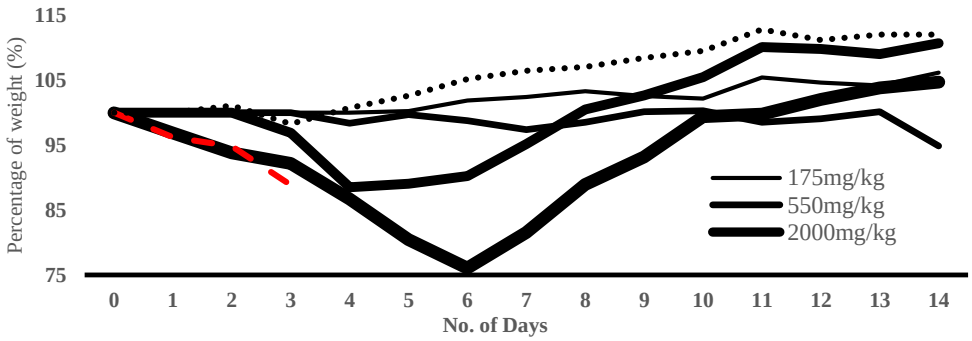

Figure 4. 2: Acute toxicity study: Body weight of animals

The body weight of the study animals were expressed in percentage is shown in Figure 4.2. The control and 175 mg/kg dose administered animal maintains an average of 13% and 5% respectively. The 550 mg/kg dosed animal maintains till the last with minimum reduction of 2-3%. The 2000 mg/kg dosed animal shows decline with 10-25% up to day 6 and gradually increases with 5-10%. An animal of 2000 mg/kg dose died on day3 with a reduction of 10% in body weight.

Organ weight:

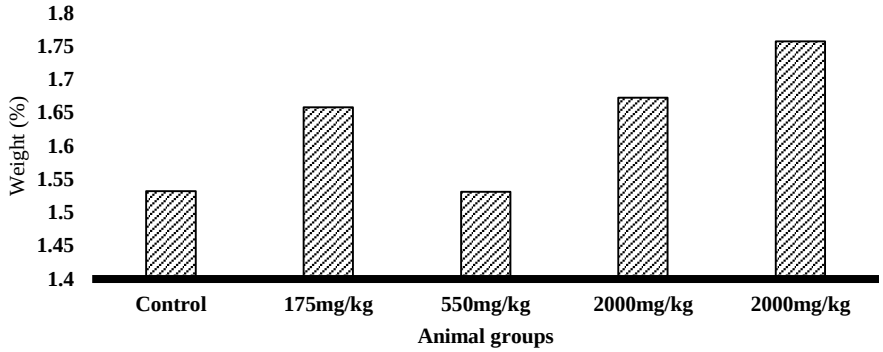

Figure 4. 3: The weight of brain of acute toxicity study animals in percentage.

The weight of brain of the study animals with respect to their body weight is shown in Figure 4.3. There is no significance noted when compared with control because of the sample size (n=1).

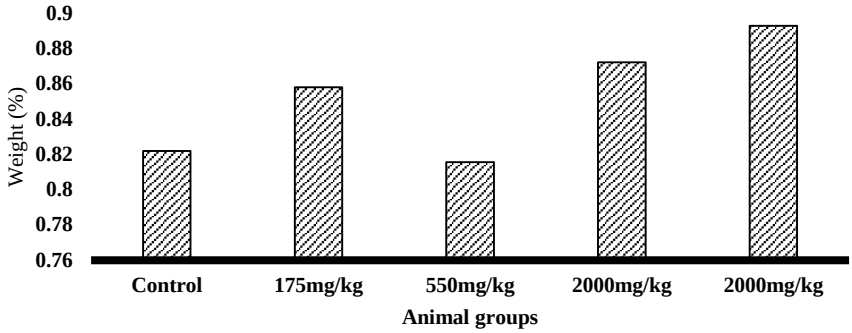

Figure 4. 4: The weight of heart of acute toxicity study animals in percentage.

The weight of heart of the study animals with respect to their body weight is shown in Figure 4.4. There is no significance noted when compared with control because of the sample size (n=1).

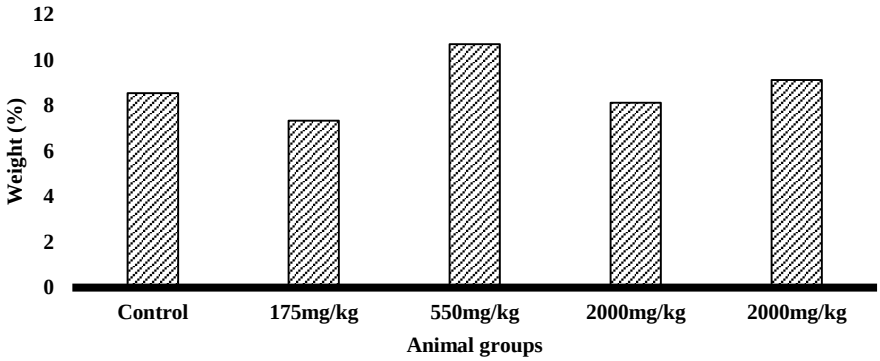

Figure 4. 5: The weight of liver of acute toxicity study animals in percentage.

The weight of liver of the study animals with respect to their body weight is shown in Figure 4.5. There is no significance noted when compared with control because of the sample size (n=1).

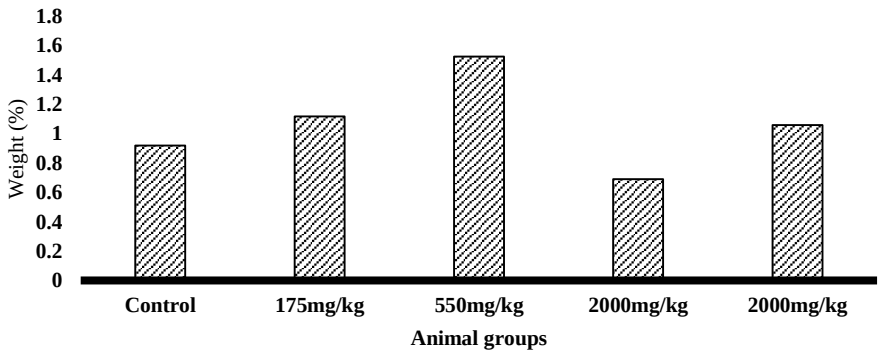

Figure 4. 6: The weight of spleen of acute toxicity study animals in percentage.

The weight of spleen of the study animals with respect to their body weight is shown in Figure 4.6. There is no significance noted when compared with control because of the sample size (n=1).

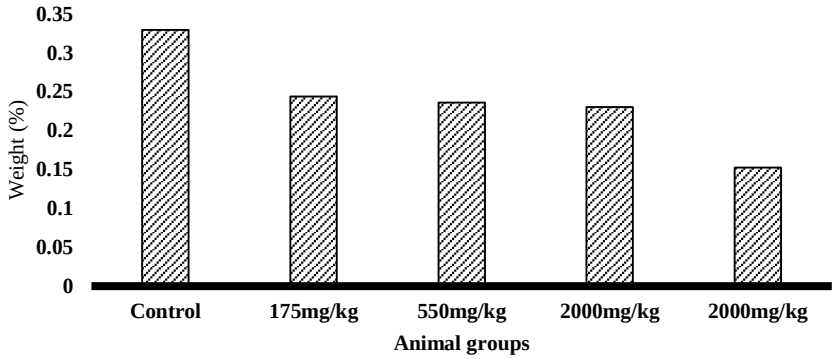

Figure 4. 7: The weight of thymus of acute toxicity study animals in percentage.

The weight of thymus of the study animals with respect to their body weight is shown in Figure 4.7. There is no significance noted when compared with control because of the sample size (n=1).

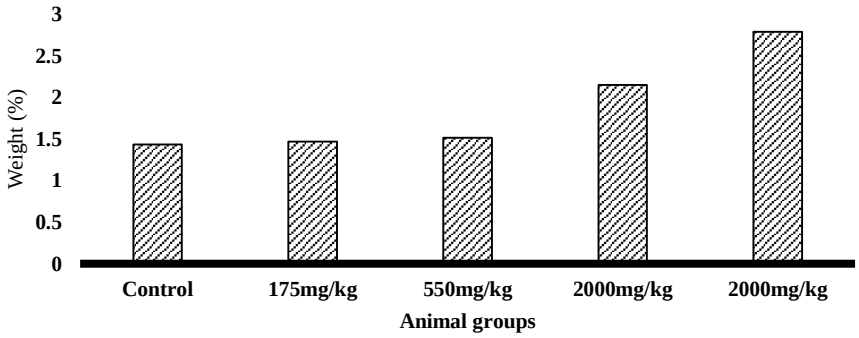

Figure 4. 8: The weight of kidney of acute toxicity study animals in percentage.

The weight of kidney of the study animals with respect to their body weight is shown in Figure 4.8. There is no significance noted when compared with control because of the sample size (n=1).

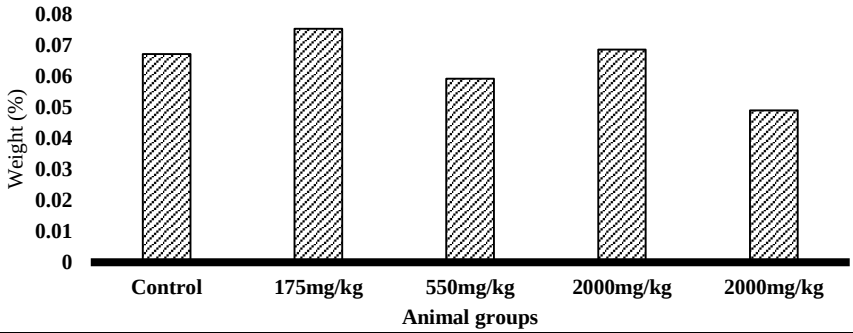

Figure 4. 9: The weight of adrenal gland of acute toxicity study animals in percentage.

The weight of adrenal gland of the study animals with respect to their body weight is shown in Figure 4.9. There is no significance noted when compared with control because of the sample size (n=1).

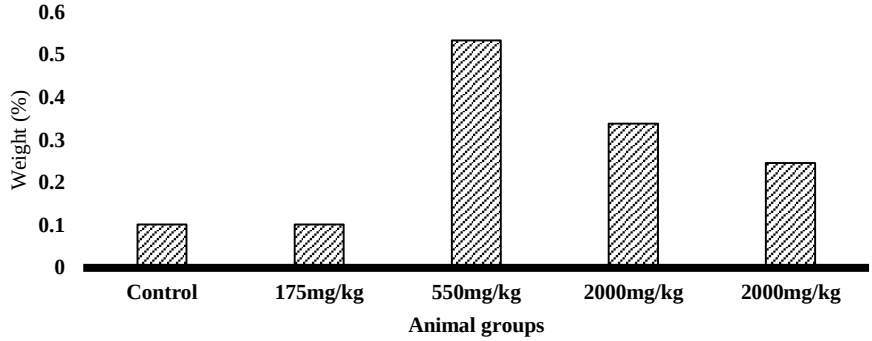

Figure 4. 10: The weight of uterus of acute toxicity study animals in percentage.

The weight of uterus of the study animals with respect to their body weight is shown in Figure 4.10. There is no significance noted when compared with control because of the sample size (n=1).

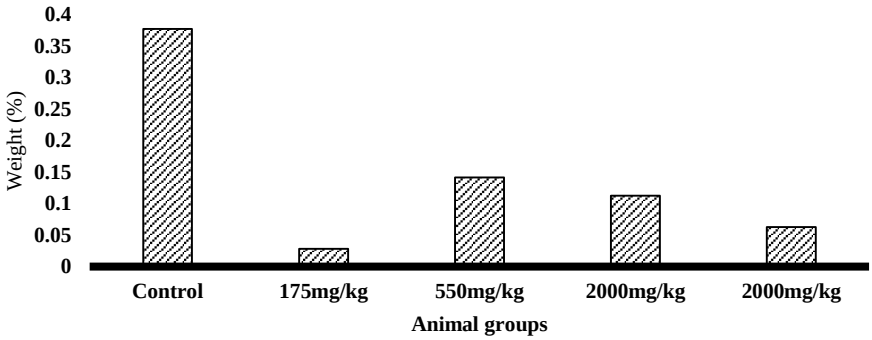

Figure 4. 11: The weight of ovary of acute toxicity study animals in percentage.

The weight of ovary of the study animals with respect to their body weight is shown in Figure 4.11. There is no significance noted when compared with control because of the sample size (n=1).

Gross pathology:

An animal of 2000 mg/kg dose showed significant reduction in spleen size. All the three animals administered with 2000mg/kg showed rough impressions on the surface of kidney. Other than this observation, no changes were noted in gross pathology.

Sub-Acute Toxicity:

Mortality and Morbidity:

Among the 70 animals, 13 animals were dead before the completion of the study. Among the 13 animals 12 were mortal and 1 was moribund. The death details are given in table 4.2.

Table 4. 2 Intermediate death details of the animals in 28 days repeated oral toxicity study.

Day of death	Group of dead animal	Sex	Death details
3	Satellite high dose	Male	Mortal
4	High dose	Male	Mortal
4	High dose	Male	Mortal
4	Satellite high dose	Male	Mortal
4	Satellite high dose	Male	Morbid
4	Satellite high dose	Male	Morbid
4	Satellite high dose	Male	Mortal
4	High dose	Male	Mortal
4	High dose	Male	Mortal
4	High dose	Female	Morbid
6	Satellite high dose	Female	Mortal
8	Medium dose	Male	Mortal
12	Satellite high dose	Female	Morbid

Cage side observations:

Various abnormal observations were noted in animals treated with various doses of PCM. They are depicted below.

Pin point pupil:

All the animals that died in between the study period showed pin point pupil for a few hours before death. Pin point pupil was more expressed in male animals compared with female animals. However, this could not be noticed in all other animals that survived the entire study period. (Shown in fig. 4.12).

Intentional tremor:

All the animals that died in between the study period showed intentional tremor for a period of few hours to few days before death. Tremor was more expressed in male animals compared with female animals. However, tremor could not be noticed in all other animals that survived the entire study period.

Figure 4. 12: A high dose treated female animal showing pin point pupil before death

Gait:

Moreover, animals were partially paralysed when they started expressing tremors. A typical tip toe walking pattern is observed in all the animals before death (shown in fig.4.13).

Figure 4. 13: high dose treated female animal showing tiptoe walking before death

Piloerection:

All the animals that died in between the study period showed piloerection from one day after drug administration.Piloerection was equally expressed in male animals and female animals. Moreover, all the animals of medium drug treated groups showed Piloerection up to two weeks and gradually recovered after that. Piloerection could not be noticed in all other groups (low dose, vehicle control and normal control) that survived the entire study period. (Shown in fig. 4.14).

Figure 4. 14: A high dose treated female animal showing piloerection

Figure 4. 15: A medium dose treated male animal showing testicular hypertrophy

Testicular hypertrophy:

Two animals of the medium dose treated male animals showed testicular hypertrophy from 6-8 days that recovered within 2 days. However, Testicular hypertrophy could not

be noticed in mortal/moribund animals and all other group animals that survived the entire study period. (Shown in fig. 4.15)

Figure 4. 16: A medium dose treated male animal showing splenomegaly

Splenomegaly:

Splenomegaly is not observed in any of the mortal/moribund animals. But it is very prominent in all other high dose and medium dose treated male and female animals that survived the entire study period during second week of the study. Splenomegaly recovered in 3-4 days in all the affected animals. Low dose, vehicle control and high dose treated animals did not show any splenomegaly. (shown in figure.4.16).

The abnormal behavioural changes were observed and graphed in Figure 4.17 with respect to the obtained cumulative score of the study animals.

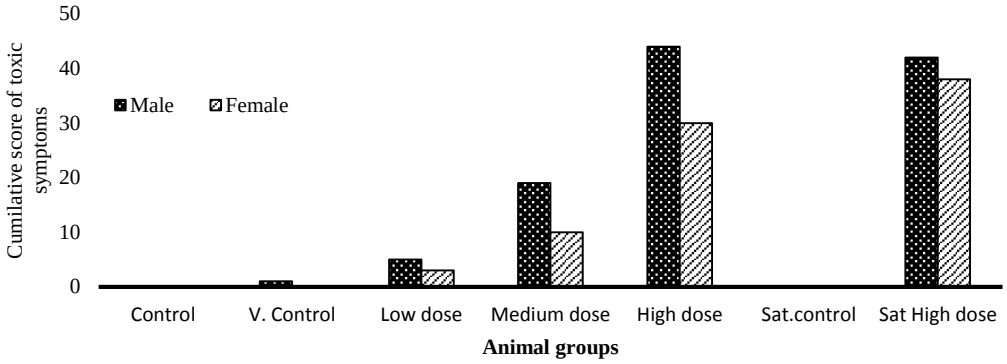

Figure 4. 17: The abnormal behavioral changes of the study animals were presented in the form of cumulative score.

Feed weight:

Feed consumption with respect to body weight of male and female animals are expressed in percentage and is shown in Figure.4.18 and figure 4.19 respectively.

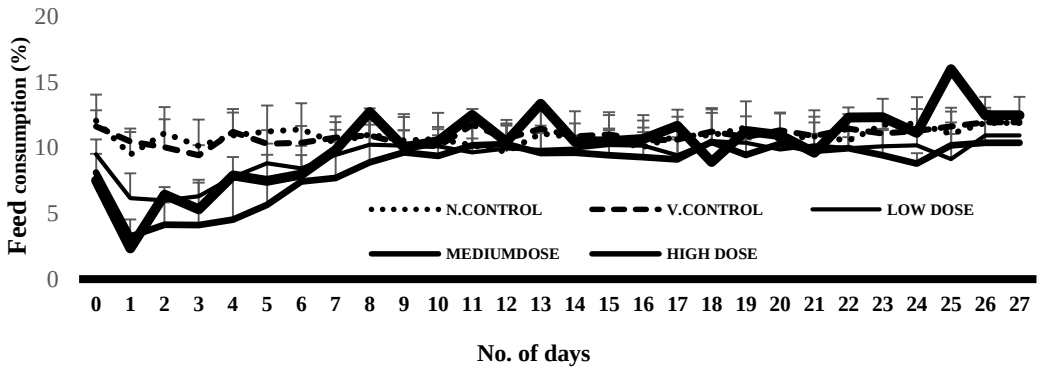

Figure 4. 18:28-repeated toxicity study: Feed consumption with respect to body weight of male animals. n=3-5 in all groups except in high dose (n=1).

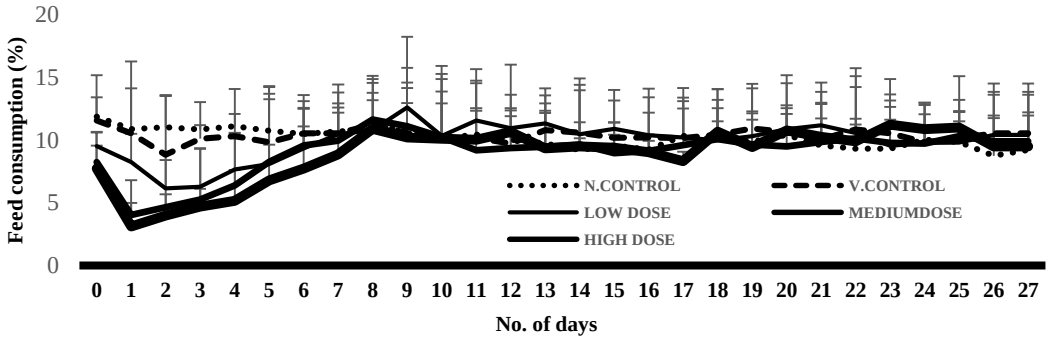

Figure 4. 19: 28-repeated toxicity study: Feed consumption with respect to body weight of femaleanimals. (n=3-5)

The average feed consumption in normal and vehicle control group range between 9-12percentage. All other groups showed a significant decline in feed consumption up to

day 8 but weresimilar to control group from day 9. But in high dose, significant difference could not made out because of low sample size (n=1).

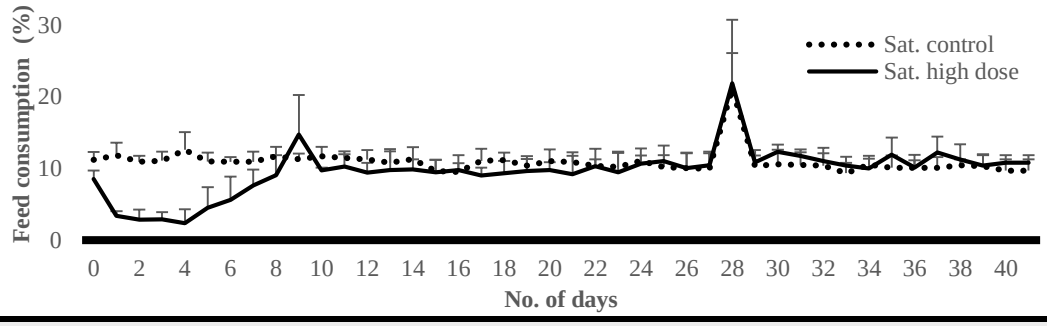

Figure 4. 20: 28-repeated toxicity study: Feed consumption with respect to bodyweight of satellite group female animals. n=3-5 in all groups.

Feed consumption with respect to body weight of satellite group animals are expressed in percentage and is shown in Figure.4.20.The feed consumption was identical to the observations in drug administered groups.

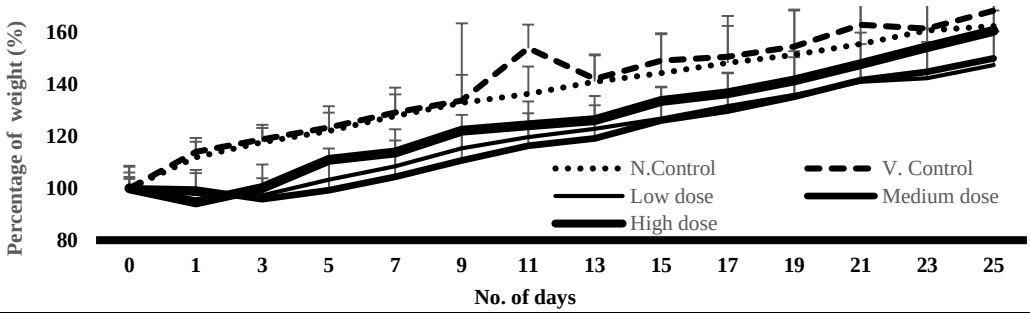

Figure 4. 21:Body weight of male animals in percentage. n=3-5 in all groups except in high dose (n=1).

The increase in body weight of male animals were expressed in percentage and is shown in Figure.4.21.

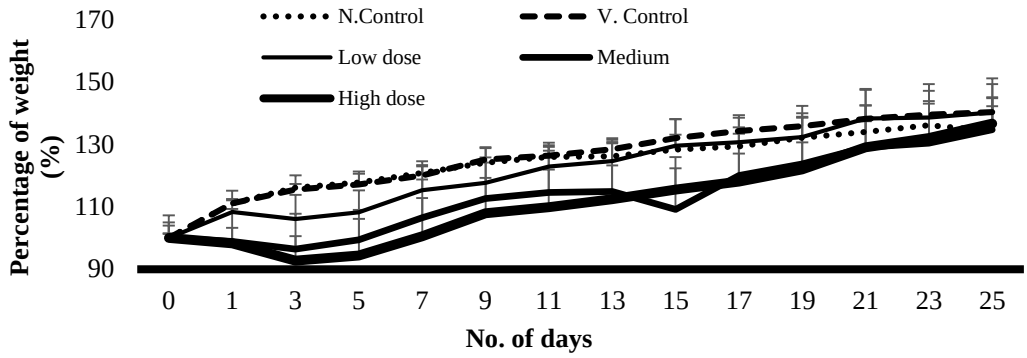

Figure 4. 22: Body weight of female animals in percentage. n=3-5 in all groups.

Thenormal and vehicle control group shows an increase of 60-65% from initial body weight. In low and medium dose groups, there is an initial reduction till day 3 followed by gradual increase up to 45%. In high dose, one animal survives till last day of study and shows reduction in weight initially followed by an increase up to 60%.

The increase in body weight of female animals was expressed in percentage and is shown in Figure. 4.22. Thenormal and vehicle control group shows an increase of 35-40% from initial body weight. In low dose group, there is a gradual increase from the initial day and shows 40% increase. In medium and high dose groups, there is initial reduction till day 3 followed by gradual increase up to 35%.

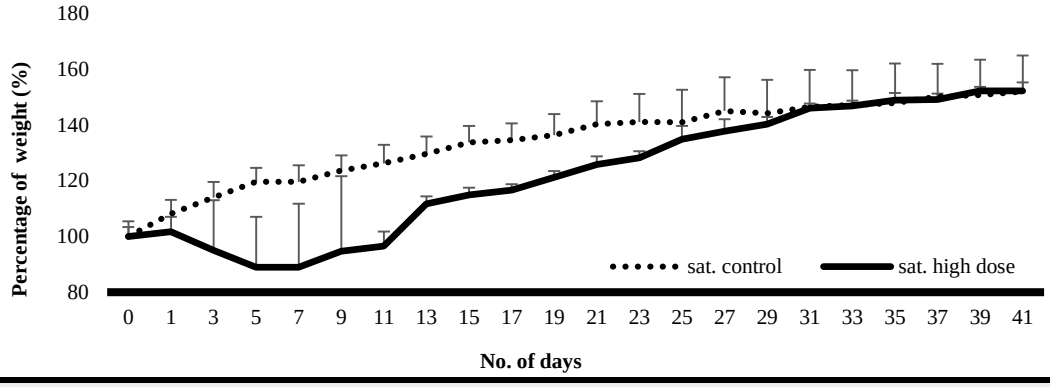

Figure 4. 23: Body weight of female animals in percentage. n=3-5 in all groups.

The increase in body weight of female satellite group animals were expressed in percentage and is shown in Figure.4.23. Thecontrol group shows an increase of 50-52% from initial body weight. In satellite high dose group, there is an initial reduction till day 5 followed by gradual increase and reaches 50% of increase at the end of study.

Organ weight:

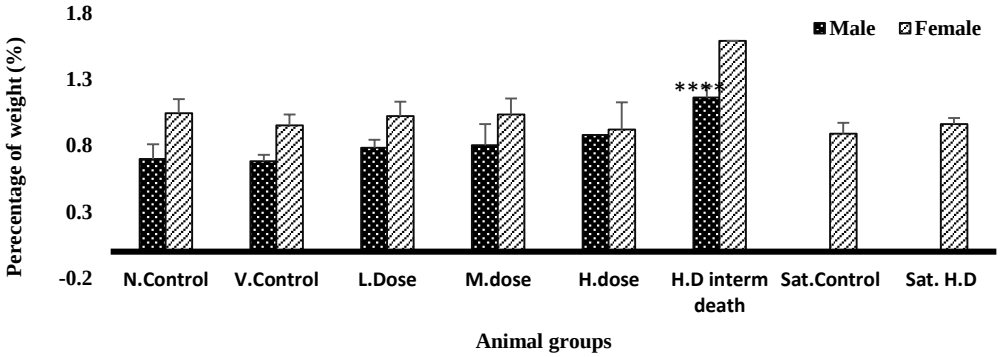

Figure 4. 24: Weight of brain of study animals with respect to the body weight. **** p <0.01. n=3-5 in all groups except Male High dose group (n=1) and Female intermediate death (n=2)

The weight of brain in male and female animals are expressed in percentage with respect to the individual body weight and is shown in Figure 4.24. There is a significant increase of 30% in male high dose intermediate death group when compared with normal control. Although the female high dose intermediate death group shows 60% increase, but the sample size is low and that the statistical significance could not be made out.

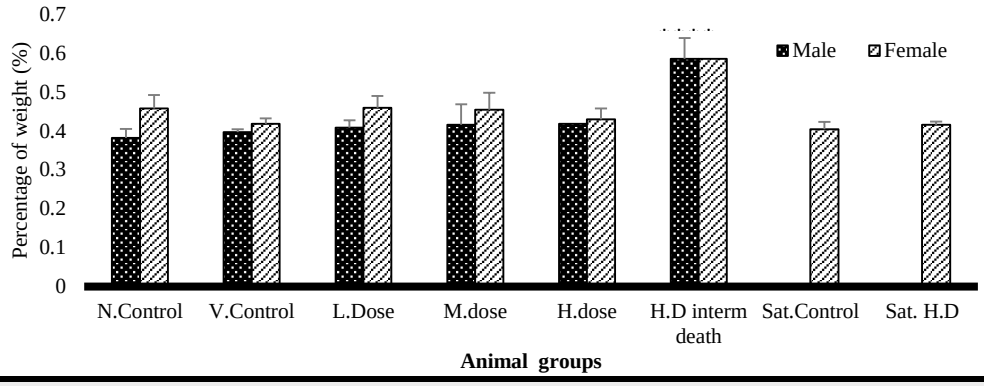

Figure 4. 25: Weight of heart of study animals with respect to the body weight. **** p <0.01. n=3-5 in all groups except Male High dose group (n=1) and Female intermediate death (n=2).

The weight of heart in male and female animals are expressed in percentage with respect to the individual body weight and is shown in Figure 4.25. There is a significant increase of 15% in male high dose intermediate death group when compared with normal control group.

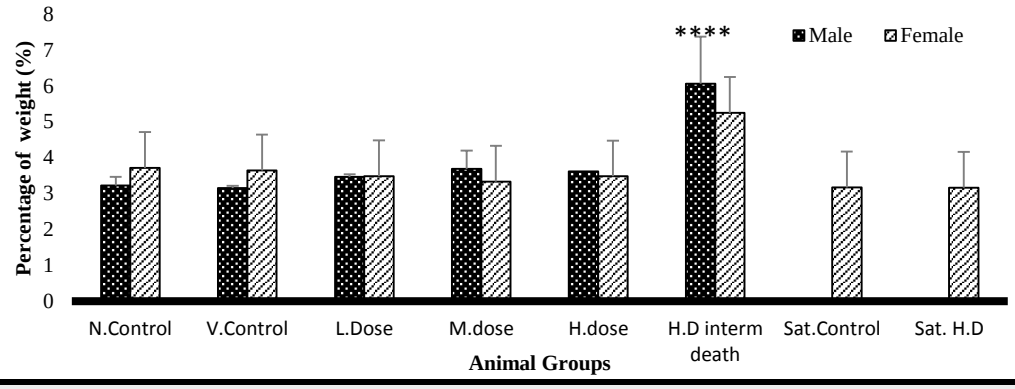

Figure 4. 26: Weight of liver of study animals with respect to the body weight. . **** p <0.01. n=3-5 in all groups except Male High dose group (n=1) and Female intermediate death (n=2)

The weight of liver in male and female animal groups are expressed in percentage with respect to the individual body weight is shown in Figure 4.26. This shows that there is a significant increase in intermediate death of high and satellite high dose treated male animals.

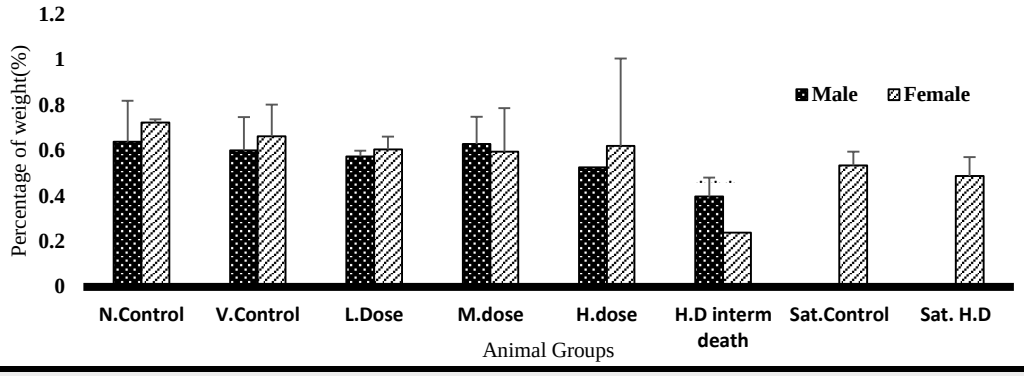

Figure 4. 27: Weight of spleen of study animals with respect to the body weight. . *** p <0.05. n=3-5 in all groups except Male High dose group (n=1) and Female intermediate death (n=2).

The weight of spleen in male and female animals are expressed in percentage with respect to the individual body weight and is shown in Figure 4.27. There is a significant reduction of 20% in male high dose intermediate death group when compared to normal control group.

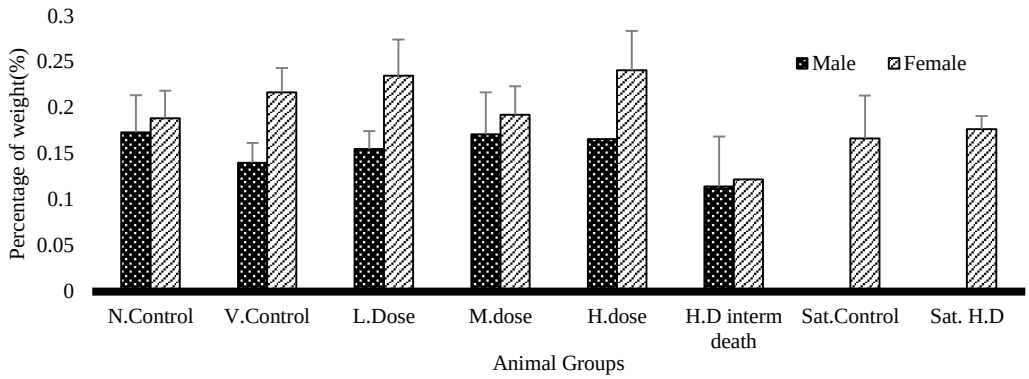

Figure 4. 28:Weight of thymus of study animals with respect to the body weight. . **** p <0.01. n=3-5 in all groups except Male High dose group (n=1) and Female intermediate death (n=2).

The weight of thymus in male and female animals are expressed in percentage with respect to the individual body weight and is shown in Figure 4.28. There is no significant difference among the groups.

The weight of kidney in male and female animals are expressed in percentage with respect to the individual body weight and is shown in Figure 4.29. There is a significant increase of 40%, 60% in male medium dose and male high dose intermediate death and 30%, 50% and 40% in female low dose, medium dose and high dose group respectively.

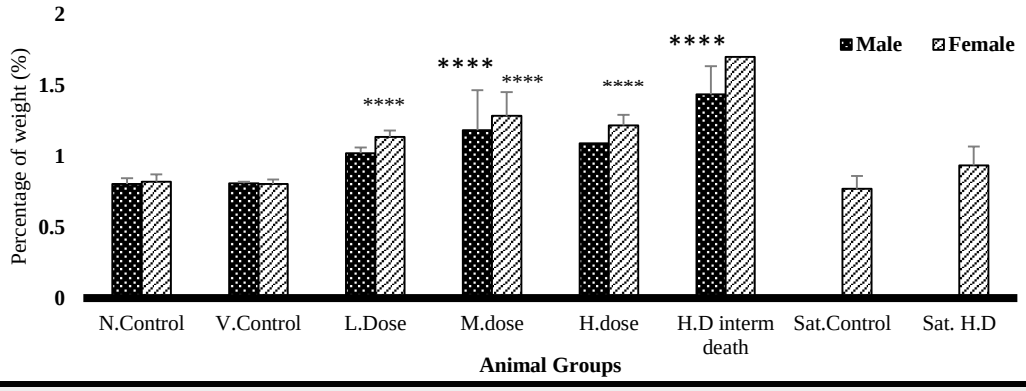

Figure 4. 29: Weight of kidney of study animals with respect to the body weight. . **** p <0.01. n=3-5 in all groups except Male High dose group (n=1) and Female intermediate death (n=2).

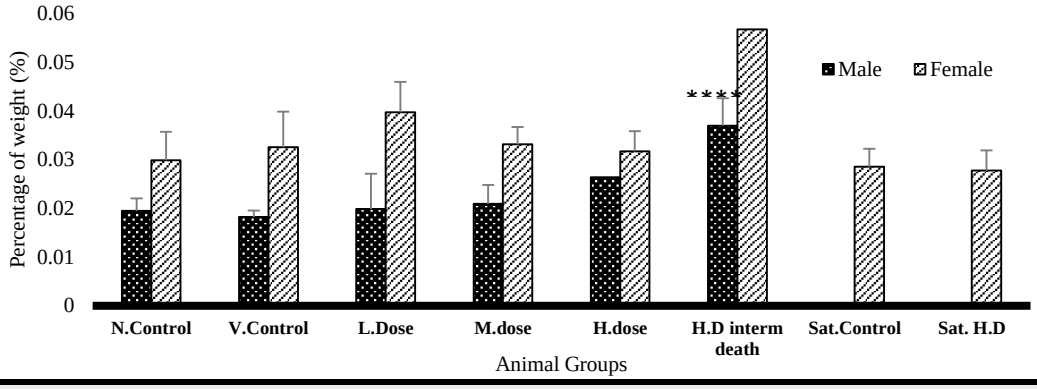

Figure 4. 30: Weight of adrenal gland of study animals with respect to the body weight. . **** p <0.01. n=3-5 in all groups except Male High dose group (n=1) and Female intermediate death (n=2).

The weight of adrenal gland in male and female animals are expressed in percentage with respect to the individual body weight and is shown in Figure 4.30. There is a significant increase of 1.5% inmale high dose intermediate death group when compared with normal control.

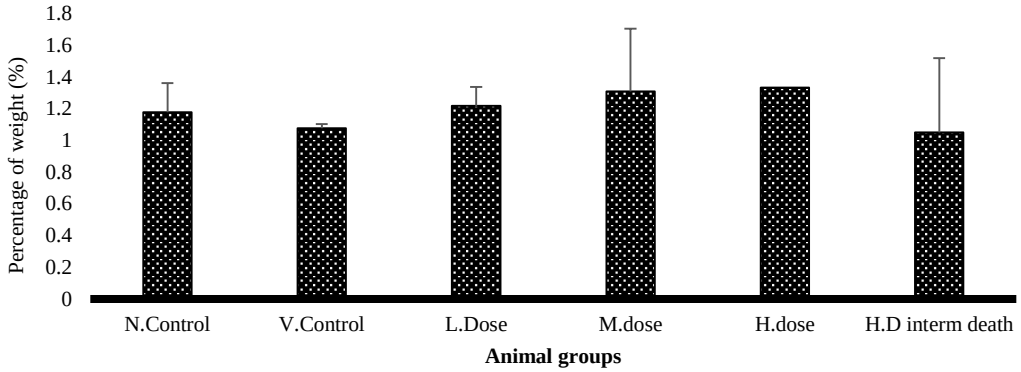

Figure 4. 31: Weight of testis of study animals with respect to the body weight. . **** p <0.01. n=3-5 in all groups except Male High dose group (n=1).

The weight of testis in male animal group are expressed in percentage with respect to the individual body weight and is shown in Figure 4.31. No significant difference is observed among the groups.

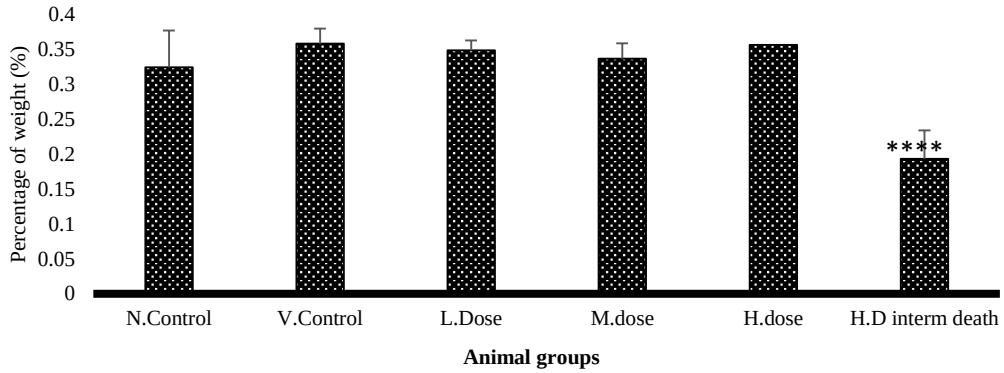

Figure 4. 32: Weight of epididymis of study animals with respect to the body weight. . **** p <0.01. n=3-5 in all groups except Male High dose group (n=1).

The weight of epididymis in male animal group are expressed in percentage with respect to the individual body weight and is shown in Figure 4.32. There is a significant 14% reduction in male high dose intermediate death group when compared with normal control.

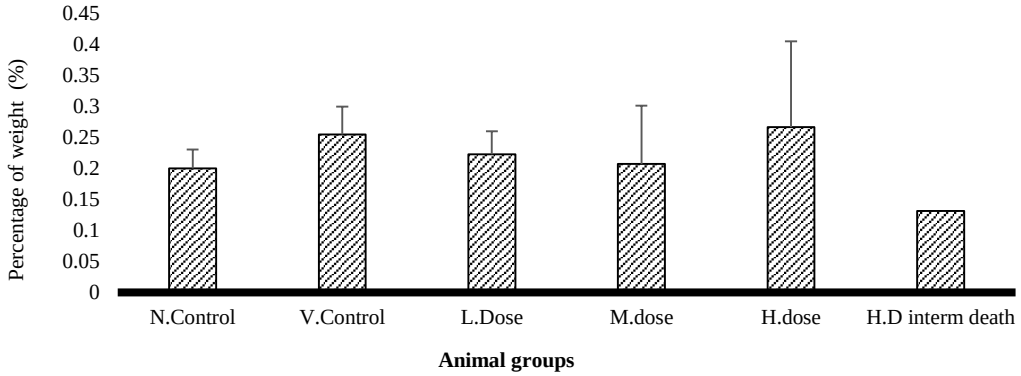

Figure 4. 33: Weight of uterus of study animals with respect to the body weight. . **** p <0.01. n=3-5 in all groups except High dose intermediate death group (n=2).

The weight of uterus in female animals are expressed in percentage with respect to the individual body weight and is shown in Figure 4.33. There is no significant difference between groups.

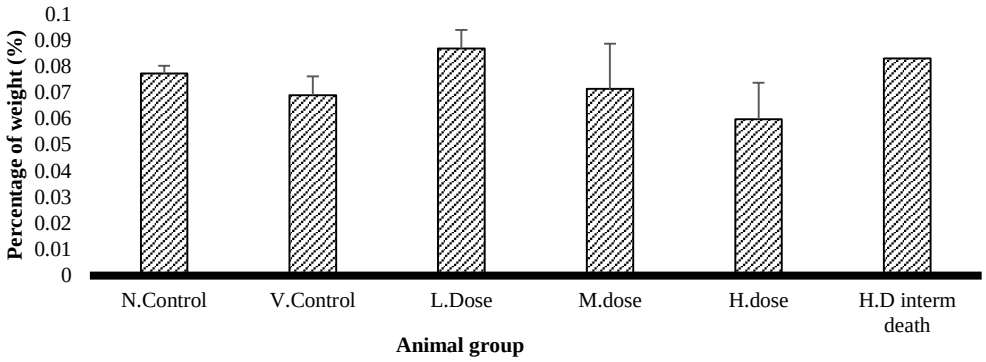

Figure 4. 34: Weight of ovary of study animals with respect to the body weight. . **** p <0.01. n=3-5 in all groups except High dose intermediate death group (n=2).

The weight of ovary in female animals are expressed in percentage with respect to the individual body weight and is shown in Figure 4.34. There is no significant difference between groups.

Haematology Analysis:

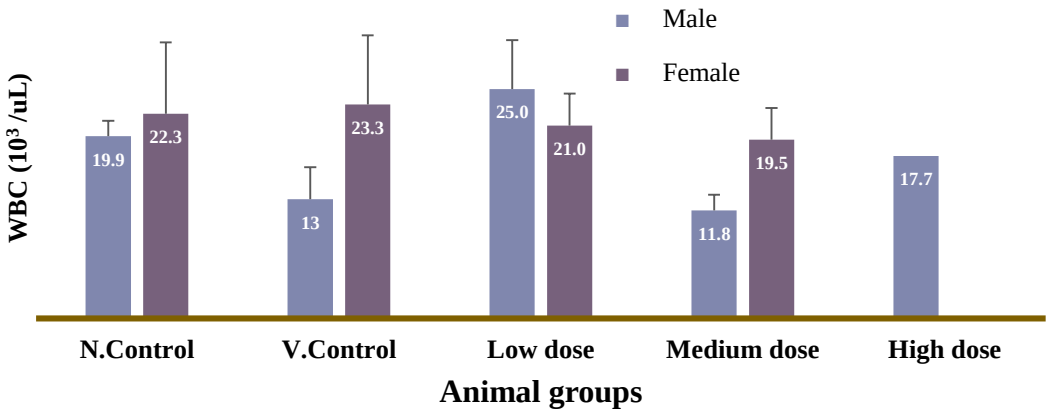

Figure 4. 35: The effect of PCM on WBC in 28-days repeated oral toxicity study animals.

The total count of white blood cells in 28-days repeated oral toxicitystudy animals is shown in Figure 4.35. No significant difference could be noted in any groups compared with normal control.

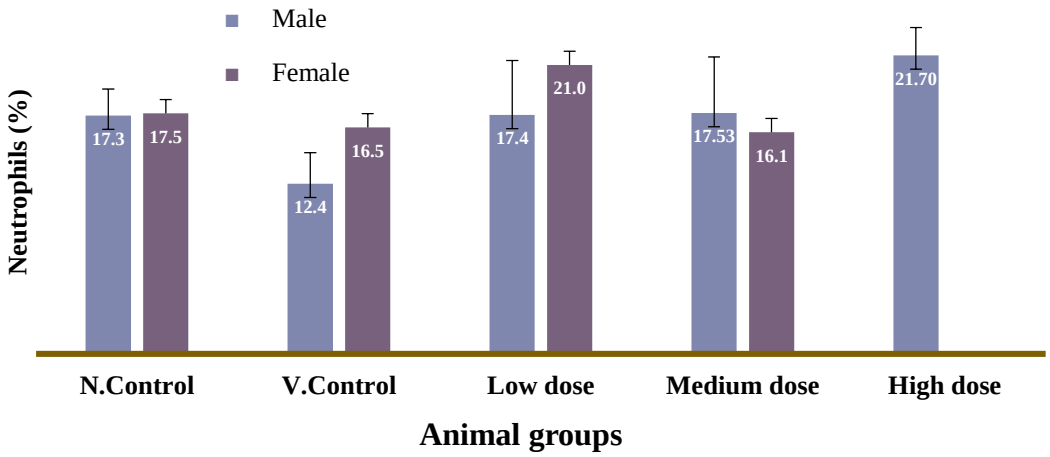

Figure 4. 36: The effect of PCM on Neutrophil in 28-days repeated oral toxicity study animals.

The percentage of neutrophil in 28-days repeated oral toxicitystudy animals is shown in Figure 4. 36. No significant difference could be noted in any groups compared with normal control.

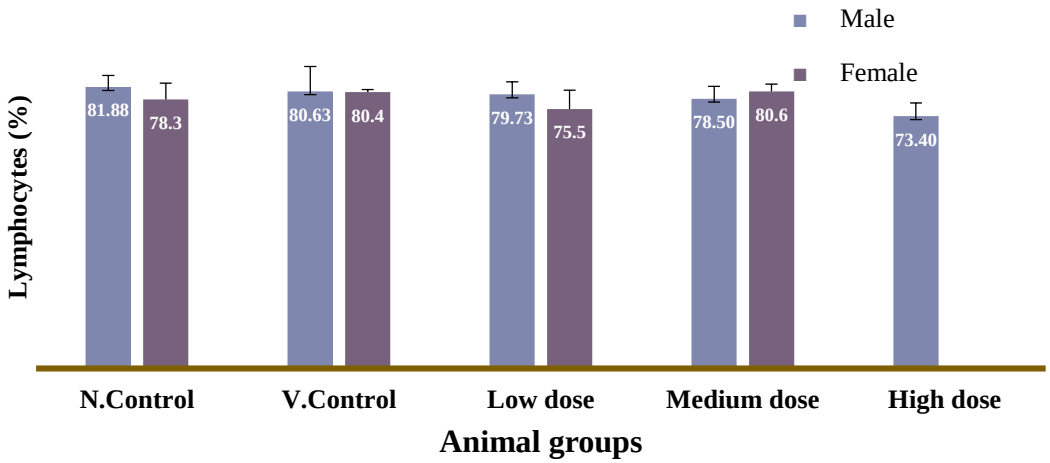

Figure 4. 37: The effect of PCM on Lymphocytes in 28-days repeated oral toxicity study animals.

The percentage of lymphocytes in 28-days repeated oral toxicitystudy animals is shown in Figure 4. 37. No significant difference could be noted in any groups compared with normal control.

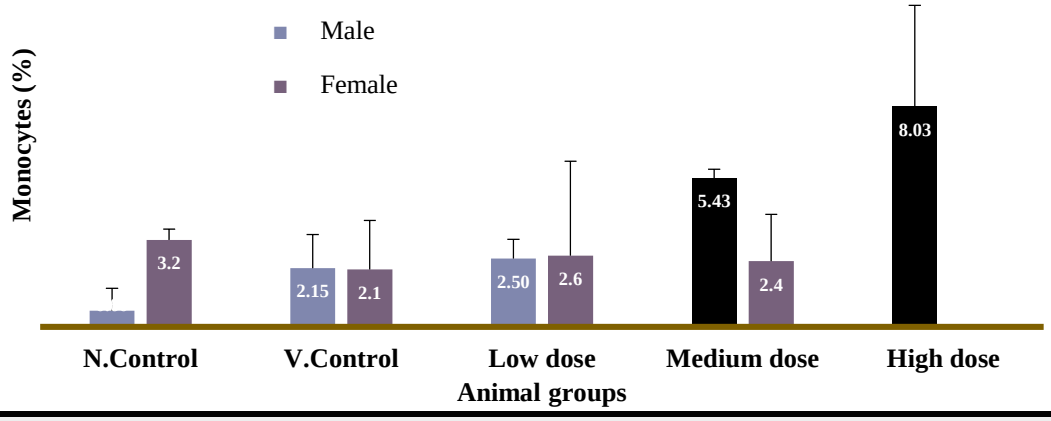

Figure 4. 38: The effect of PCM on Monocytes in 28-days repeated oral toxicity study animals.

The percentage of monocytes in 28-days repeated oral toxicitystudy animals is shown in Figure 4. 38. Monocyte count was significantly increased in medium dose and high dose treated male animals. No significant difference could be noted in any groups compared with normal control.

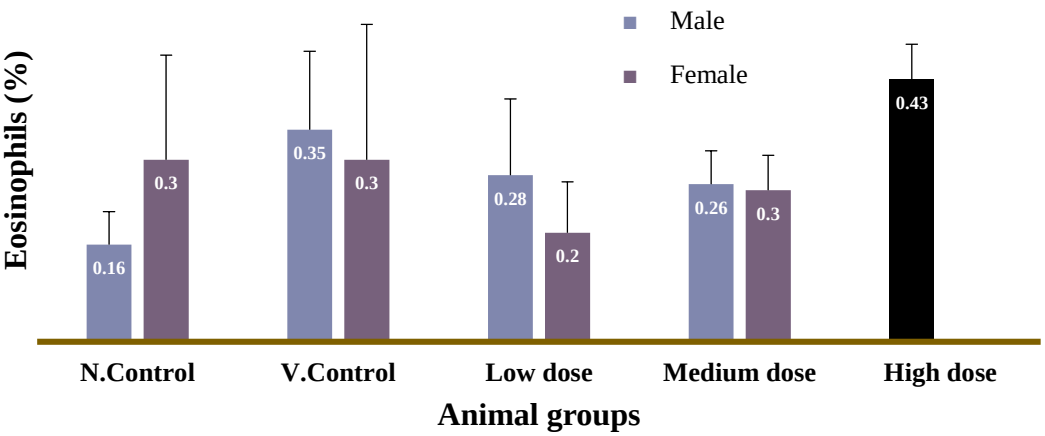

Figure 4. 39: The effect of PCM on Eosinophil in 28-days repeated oral toxicity study animals.

The percentage of Eosinophil in 28-days repeated oral toxicitystudy animals is shown in Figure 4. 39. Eosinophil count was significantly increased in high dose treated male animals. No significant difference could be noted in any other groups compared with normal control.

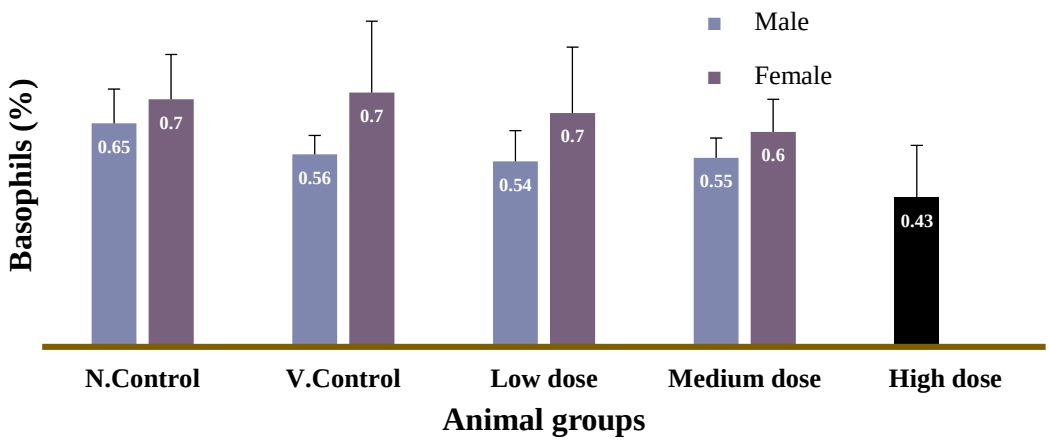

Figure 4. 40: The effect of PCM on Basophil in 28-days repeated oral toxicity study animals.

The percentage of Basophil in 28-days repeated oral toxicitystudy animals is shown in Figure 4. 40. Basophil count was significantly increased in high dose treated male animals. No significant difference could be noted in any other groups compared with normal control.

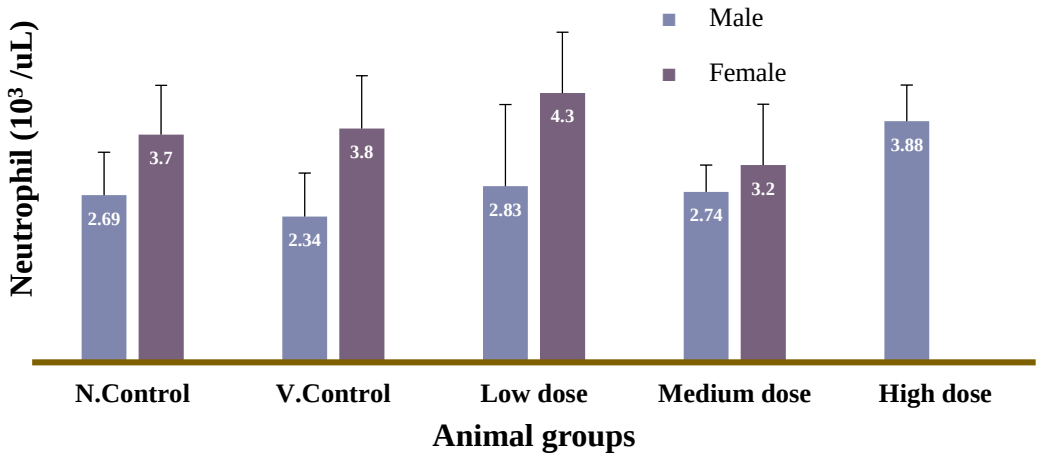

Figure 4. 41: The effect of PCM on Neutrophil in 28-days repeated oral toxicity study animals.

The total count of Neutrophil in 28-days repeated oral toxicitystudy animals is shown in Figure 4. 41. No significant difference could be noted in any other groups compared with normal control.

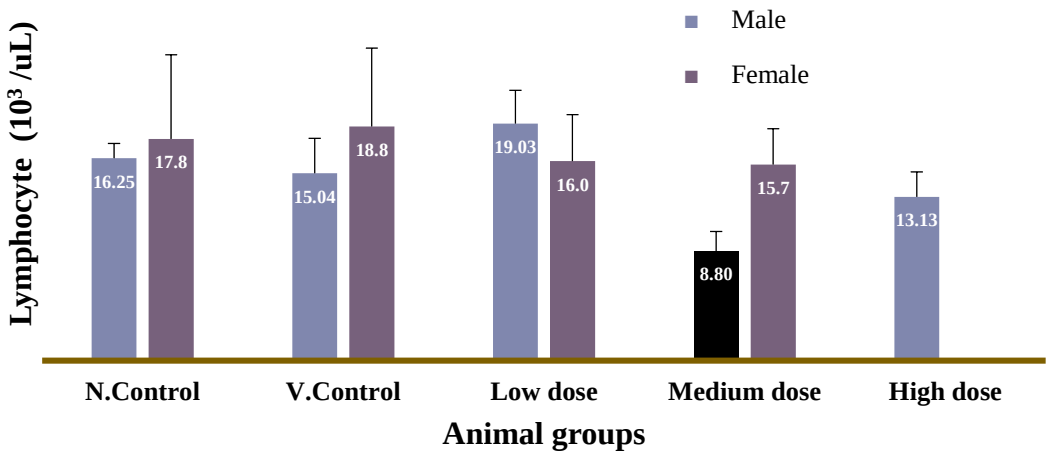

Figure 4. 42: The effect of PCM on Lymphocyte in 28-days repeated oral toxicity study animals.

The total count of Lymphocyte in 28-days repeated oral toxicity study animals is shown in Figure 4. 42. Lymphocyte count was significantly decreased in medium dose treated male animals. No significant difference could be noted in any other groups compared with normal control.

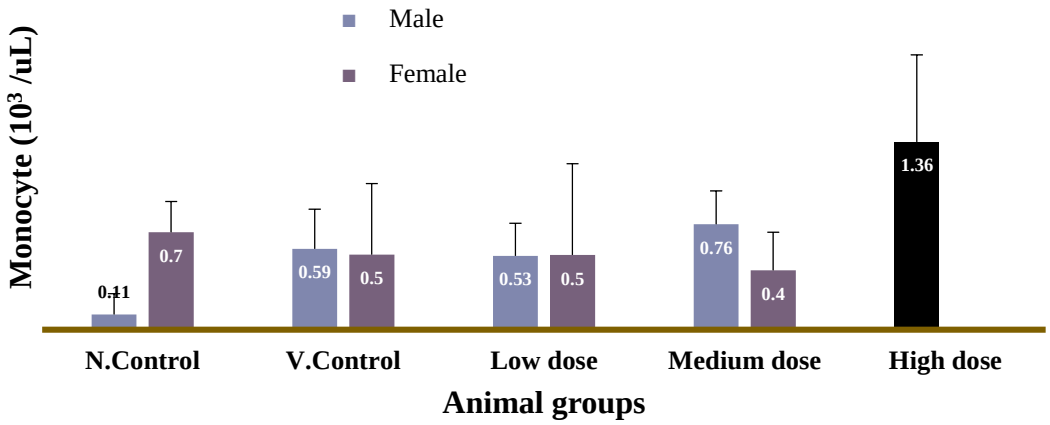

Figure 4. 43: The effect of PCM on Monocyte in 28-days repeated oral toxicity study animals.

The total amount of Lymphocyte in 28-days repeated oral toxicity study animals is shown in Figure 4. 43. Lymphocyte count was significantly decreased in medium dose treated male animals. No significant difference could be noted in any other groups compared with normal control.

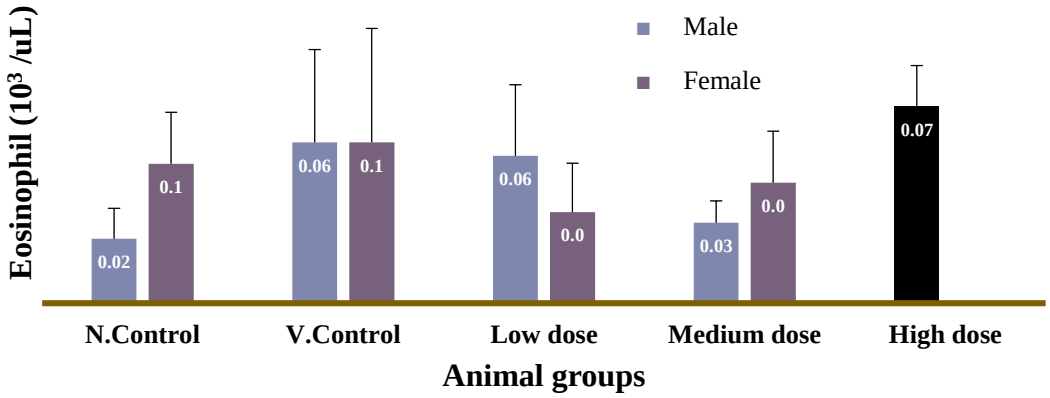

Figure 4. 44: The effect of PCM on Eosinophil in 28-days repeated oral toxicity study animals.

The total count of Eosinophil in 28-days repeated oral toxicity study animals is shown in Figure 4. 44. Eosinophil count was significantly increased in dose treated male animals. No significant difference could be noted in any other groups compared with normal control.

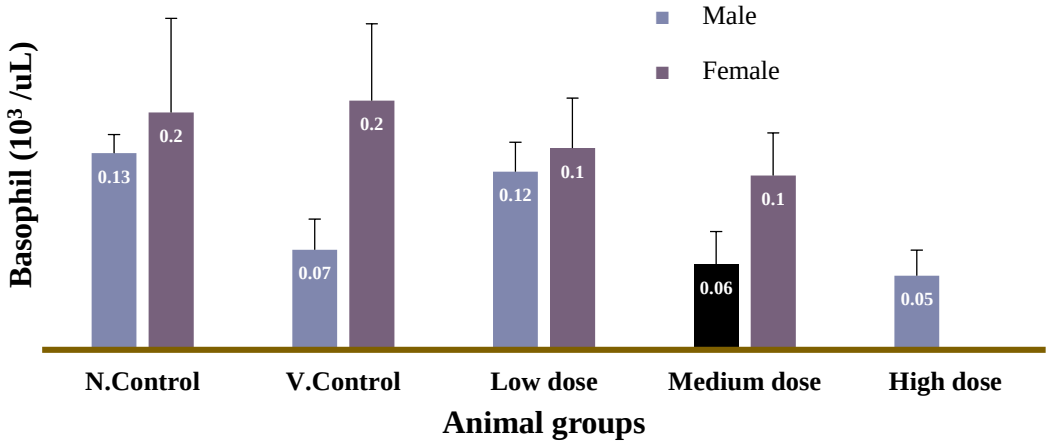

Figure 4. 45: The effect of PCM on Basophil in 28-days repeated oral toxicity study animals.

The total count of Basophil in 28-days repeated oral toxicitystudy animals is shown in Figure 4.45. Basophil count was significantly decreased in medium dose treated male animals. No significant difference could be noted in any other groups compared with normal control.

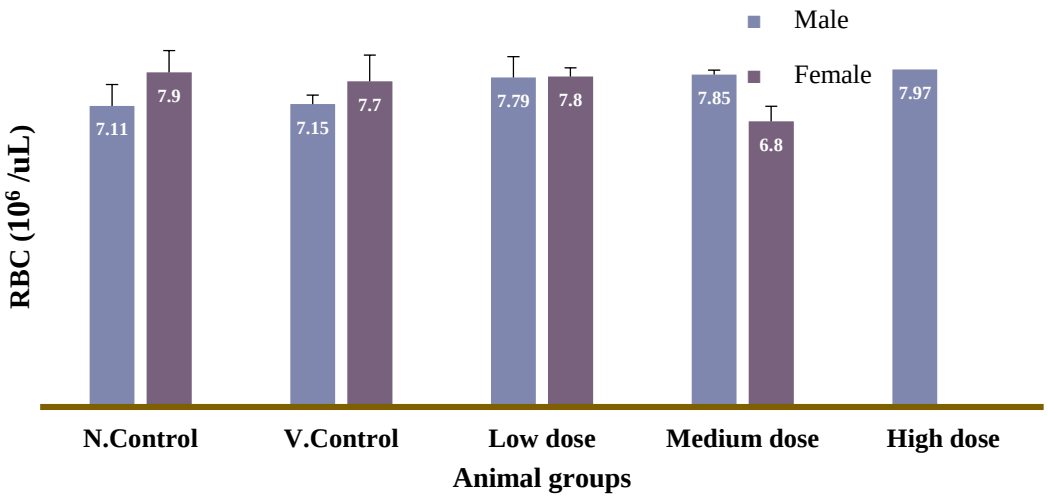

Figure 4. 46: The effect of PCM on RBC in 28-days repeated oral toxicity study animals.

The total count of Red blood cells in 28-days repeated oral toxicity study animals is shown in Figure 4.46. No significant difference could be noted in any groups compared with normal control.

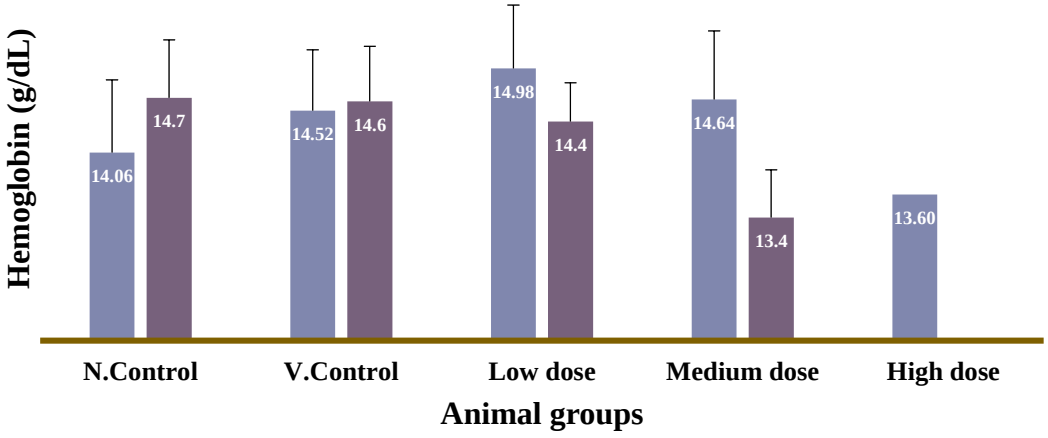

Figure 4. 47: The effect of PCM on Hemoglobin in 28-days repeated oral toxicity study animals.

The total amount of Hemoglobin 28-days repeated oral toxicity is study animals shown in Figure 4.47. No significant difference could be noted in any groups compared with normal control.

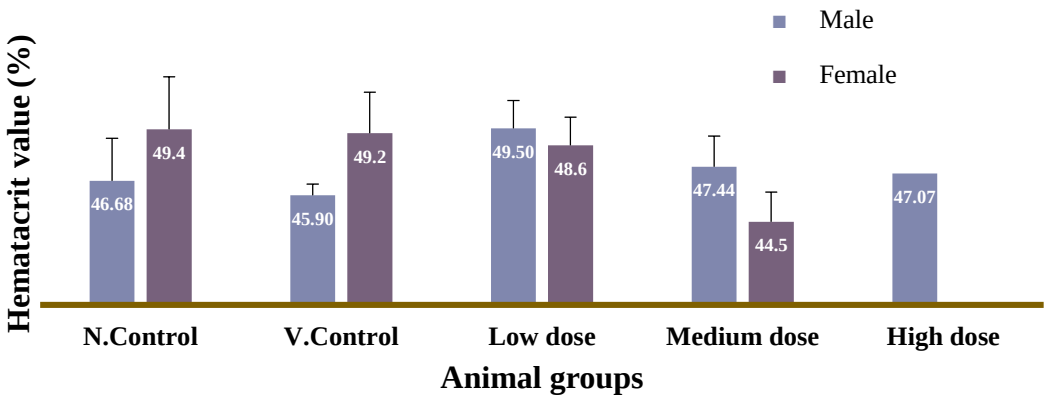

Figure 4. 48: The effect of PCM on Hematocrit value in 28-days repeated oral toxicity study animals.

The total amount of Hemoglobin in 28-days repeated oral toxicitystudy animals is shown in Figure 4.48. No significant difference could be noted in any groups compared with normal control.

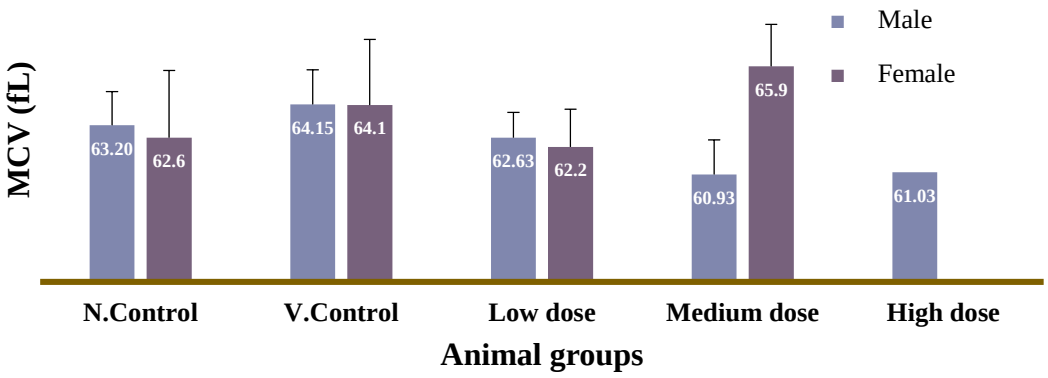

Figure 4. 49: The effect of PCM on MCV in 28-days repeated oral toxicity study animals.

The total amount of MCV in 28-days repeated oral toxicity study animals is shown in Figure 4.49. No significant difference could be noted in any groups compared with normal control.

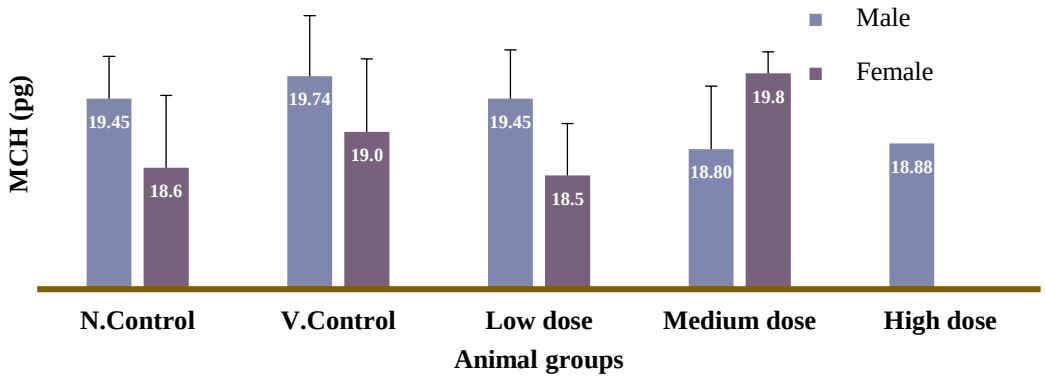

Figure 4. 50: The effect of PCM on MCH in 28-days repeated oral toxicity study animals.

The total amount of MCHin 28-days repeated oral toxicity study animals is shown in Figure 4.50. No significant difference could be noted in any groups compared with normal control.

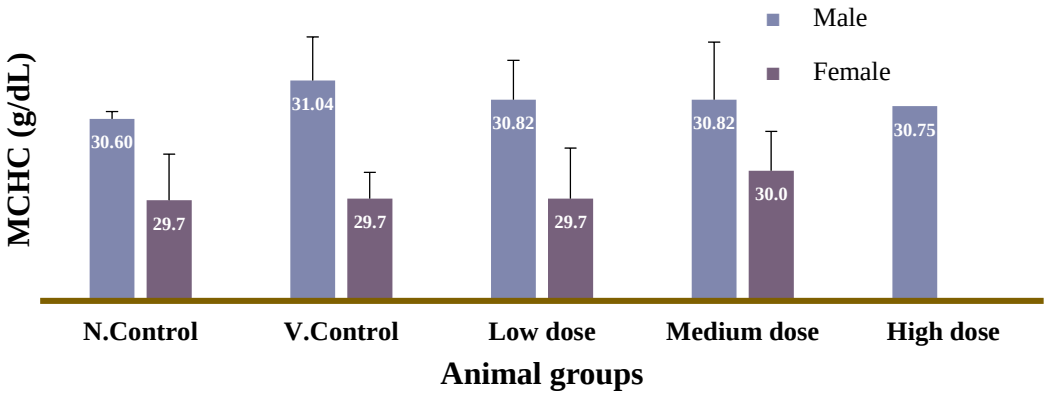

Figure 4. 51: The effect of PCM on MCHC in 28-days repeated oral toxicity study animals.

The total amount of MCHCin 28-days repeated oral toxicity study animals is shown in Figure 4.51. No significant difference could be noted in any groups compared with normal control.

The total amount of RDWCVin 28-days repeated oral toxicity study animals is shown in Figure 4.52. No significant difference could be noted in any groups compared with normal control.

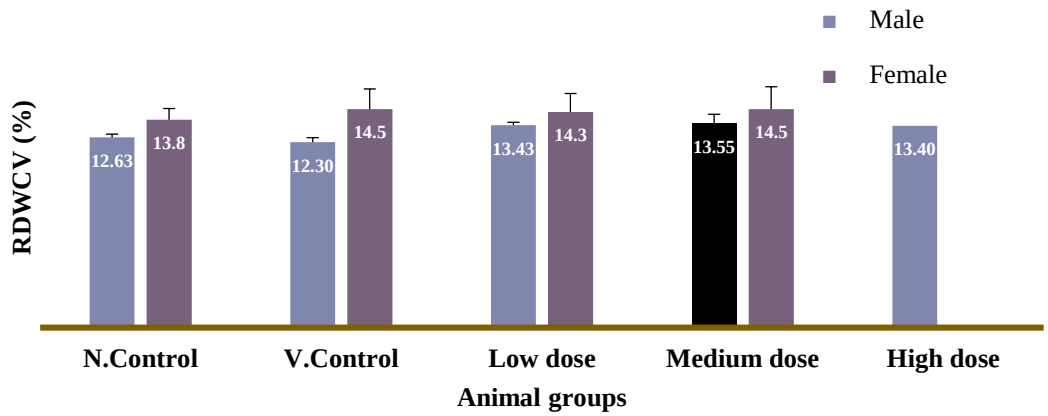

Figure 4. 52: The effect of PCM on RBC distribution width (C.V)in 28-days repeated oral toxicity study animals.

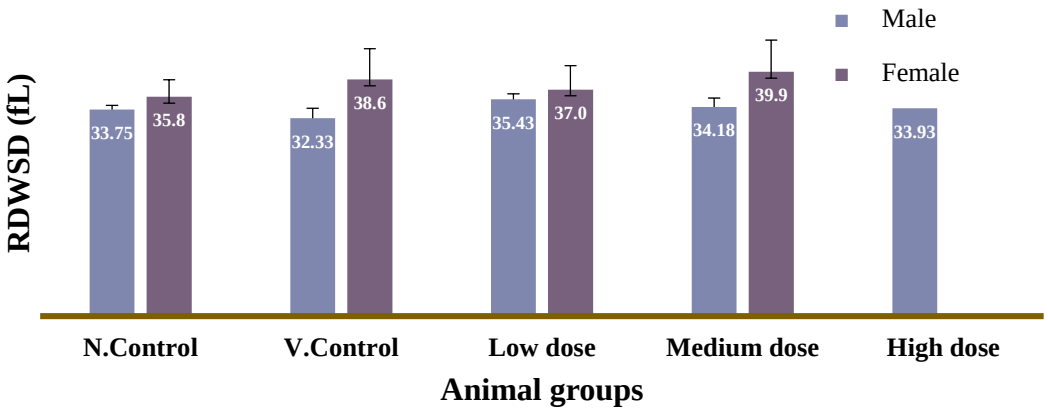

Figure 4. 53: The effect of PCM on RBC distribution width (S.D) in 28-days repeated oral toxicity study animals.

The total amount of RDWSDin 28-days repeated oral toxicity study animals is shown in Figure 4.53. No significant difference could be noted in any groups compared with normal control.

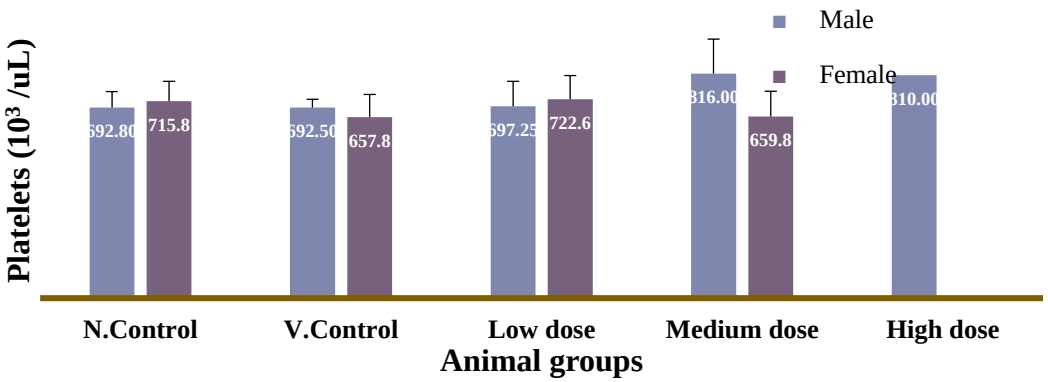

Figure 4. 54: The effect of PCM on Platelets in 28-days repeated oral toxicity study animals.

The total amount of Platelets in 28-days repeated oral toxicity study animals is shown in Figure 4.54. No significant difference could be noted in any groups compared with normal control.

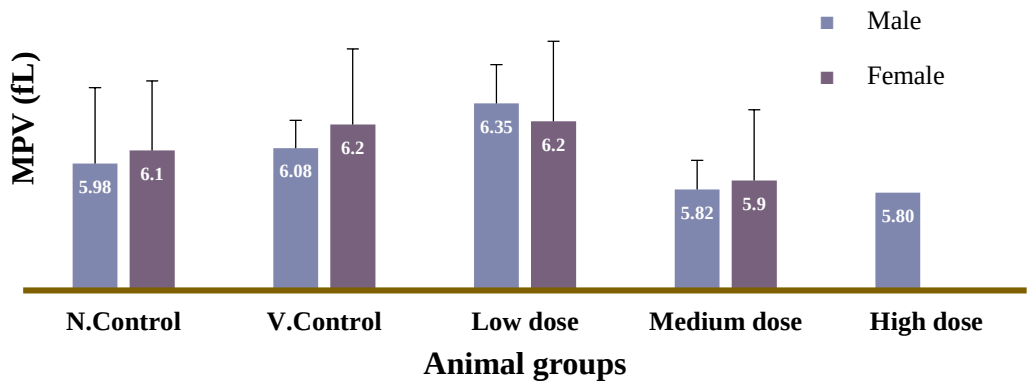

Figure 4. 55: The effect of PCM on MPV in 28-days repeated oral toxicity study animals.

The mean platelet volume in 28-days repeated oral toxicity study animals is shown in Figure 4.55. No significant difference could be noted in any groups compared with normal control.

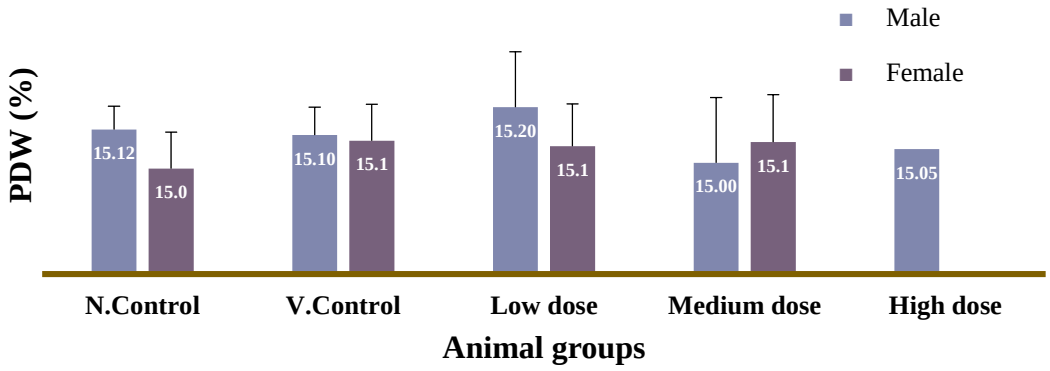

Figure 4. 56: The effect of PCM on PDW in 28-days repeated oral toxicity study animals.

The total amount of PDW in 28-days repeated oral toxicity study animals is shown in Figure 4.56. No significant difference could be noted in any groups compared with normal control.

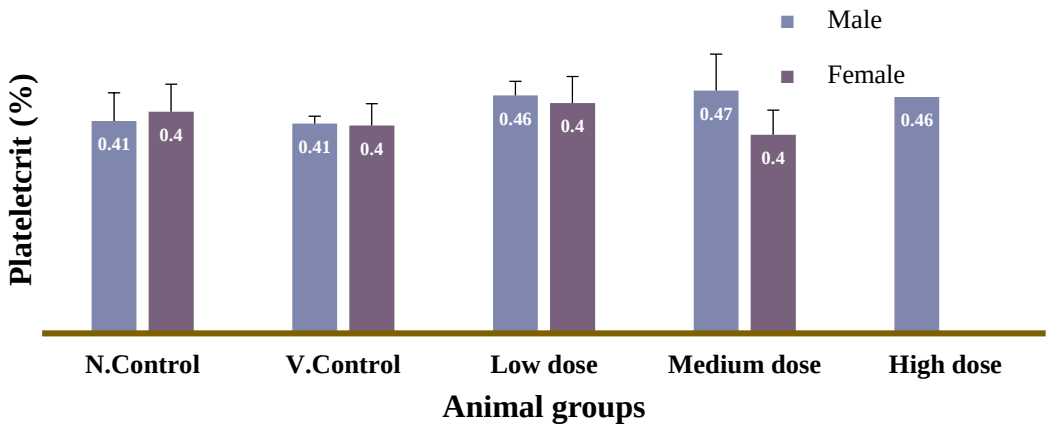

Figure 4. 57: The effect of PCM on Plateletcrit in 28-days repeated oral toxicity study animals.

The total amount of Plateletcritin 28-days repeated oral toxicity study animals is shown in Figure 4.57. No significant difference could be noted in any groups compared with normal control.

Bio-chemical Analysis:

Effect of PCM on various bio-chemical parameters that regulate liver and renal functions is shown in table 4.3, table 4.4, table 4.5 and table 4.6. Bio-chemical analysis could not be performed in mortal and moribund animals.

Table 4. 3: The effect of PCM on various liver function parameters in male animals of 28 days repeated oral toxicity study. n = 1 in high dose and 4-5 in all other groups ; *P<0.05

Group	SGOT	ALP	SGPT	T.billirubin	D.bilirubin	BUN
Control	21.5±4.4	111.7±25.9	43.5±23.5	1.3±0.7	0.7±0.1	24.7±8.3
Vehicle control	28.1±9.9	107.25±18.6	7.2±1.8	0.4±0.2	0.5±0.2	37.9±1.0
Low dose	19.1±4.1	110±7.9	14.5±4.5	0.2±0.0	0.7±0.2	19.6±7.9
Medium dose	24.3±12.9	93.96±20.6	93.3±33.7	43.7±15.8	0.2±0.0	0.4±0.2
High dose	13.96	31.17	64.26	30.08	3.19	0.0

No significant difference was noted in the levels of SGOT, SGPT, ALP, blood urea nitrogen, total and direct bilirubin levels in all the PCM treated male animalswhen compared with the normal control group.

Table 4. 4: The effect of PCM on various renal function parameters in male animals of 28 days repeated oral toxicity study.n = 1 in high dose and 4-5 in all other groups ; *P<0.05

Group	Albumin	Cholesterol	Protein	Uric acid	Creatinine	Urea
Control	3.8 ±0.12	210.22±57.8	9.0±0.6	5.1±0.7	0.5±0.3	52.7±17.8
Vehicle control	3.4±.08	276.5±72.7	9.3±0.7	3.2±0.02	0.4±0.5	81.0±2.1
Low dose	3.6±0.08	261.8±61.7	8.0±0.1	3.4±0.3*	0.5±0.05	41.8±16.9
Medium dose	3.6±0.04	127.3±13.4	8.0±0.2	3.5±0.05*	0.5±0.05	93.3±33.7
High dose	3.2	563.6	7.6	4.8	0.4	64.3

No significant difference was noted in the levels of albumin, cholesterol, protein, uric acid, creatinine and urea levels in all the PCM treated male animals when compared with the normal control group.

Table 4. 5: The effect of PCM on various liver function parameters in female animals of 28 days repeated oral toxicity study.n = 4-5 in all other groups ; *P<0.05

Group	SGOT	ALP	BUN	SGPT	T.billirubin	D.bilirubin
Control	36.82±11.2	72.42±14.06	35.38±5.3	6.83±1.5	0.02±0.015	0.65±0.1
Vehicle control	15.20±3.5	60.5±12.9	28.14±12.4	7.21±2.1	0.023±0.003	0.31±0.2
Low dose	50.02±13.5	77.31±4.4	21.23±7.9	22.68±18.3	0.012±0.01	0.65±0.09
Medium dose	15.65±1.3	101.98±14.0	26.67±9.9	20.8±9.08	0.0240±0.01	0.21±0.2
High dose	38.01±28.8	91.67±12.1	31.75±13.7	12.8±6.4	0.27±0.009	0.29±0.08
Sat. Control	16.17±0.9	136.77±20.6	24.25±3.6	6.9±0.65	0.46±2	0.9±0.4
Sat. High dose	14.35±2.4	146.7±12	30.6±18.9	10.3±5.02	0.3±0.2	1.9

No significant difference was noted in the levels of SGOT, SGPT, ALP, blood urea nitrogen, total and direct bilirubin levels in all the PCM treated female animalswhen compared with the normal control group.

Table 4. 6: The effect of PCM on various renal function parameters in female animals of 28 days repeated oral toxicity study.n = 4-5 in all other groups ; *P<0.05

Group	Albumin	Cholesterol	Protein	Uric acid	Creatinine	Urea
Control	3.49±0.08	233.04±71.5	8.32±0.2	4.39±0.6	0.48±0.07	45.24±19.08
Vehicle control	3.52±0.03	173.06±26.7	8.52±0.1	3.36±0.1	0.58±0.08	89.28±3.1
Low dose	3.27±0.02	127.22±9.9	7.65±0.3	3.96±0.1	0.38±0.0	45.36±16.8
Medium dose	3.4±0.12	180.17±34.7	7.10±0.2	3.90±0.2	0.5±0.0	64.4±21.1
High dose	3.68±0.3	279.15±110.8	7.31±0.5	3.37±0.6	0.41±0.1	51.34±26.5
Sat. Control	12.23±3.2	141.45±2.7	12.5±0.1	3.52±0.6	0.33±0.03	51.8±7.7
Sat. High dose	17.32±2.7	129.8±9.9	9.16±0.6*	19.65±12	0.33±0.04	65.3±40.3

No significant difference was noted in the levels of albumin, cholesterol, protein, uric acid, creatinine and urea levels in all the PCM treated male animalswhen compared with the normal control group.

Gross pathology:

Mortal and moribund showed a significant reduction in size of spleen and epididymis (3 times). In most of the mortal and moribund animals inflammation in spleen and kidneys are noted.

Histopathology:

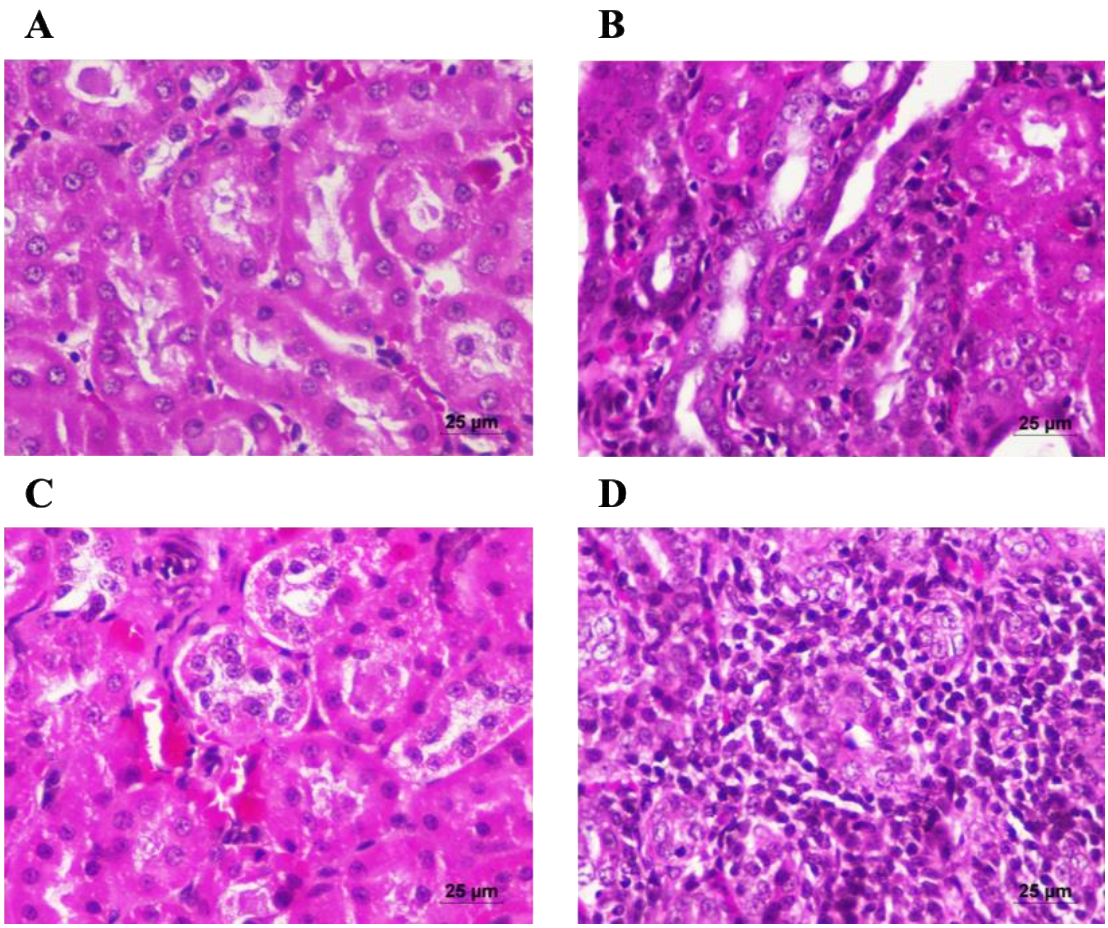

Figure 4. 58: Representative images histopathology of kidney of A) Normal control male animals B) High dose treated male animal C) Normal control female animals and D) High dose treated female animal. Images are shown at 40X magnification

Representative images of tissue sections of kidney are shown in Fig. 4.58. Marked multifocal, bilateral degeneration/necrotic changes of tubular, noted in male and female animals of high dose PCM treated group compared with normal control.

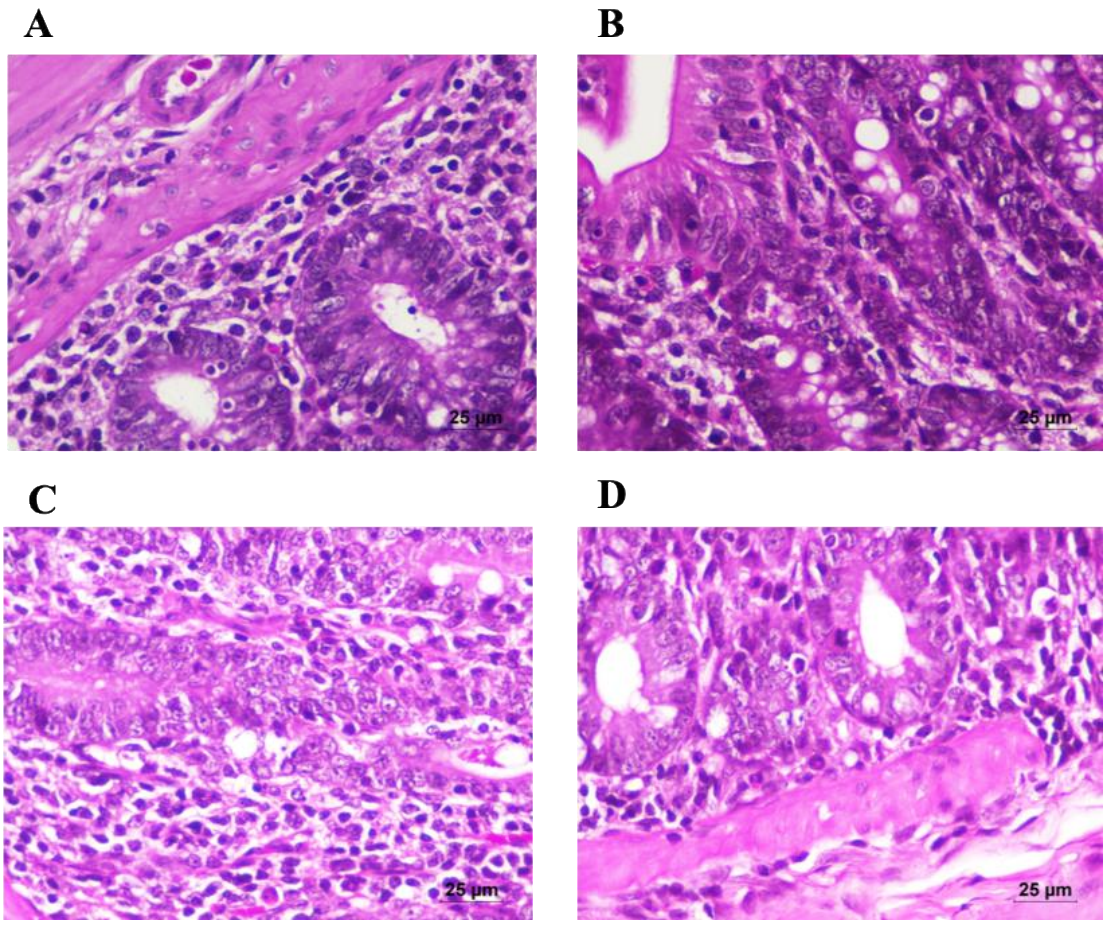

Figure 4. 59: Representative images histopathology of colon of A) Normal control male animals B) High dose treated male animal C) Normal control female animals and D) High dose treated female animal. Images are shown at 40X magnification

Representative images of tissue sections of colon are shown in Fig. 4.59. Moderate mononuclear mucosal infiltrates are noted in high dose PCM treated group compared with normal control.

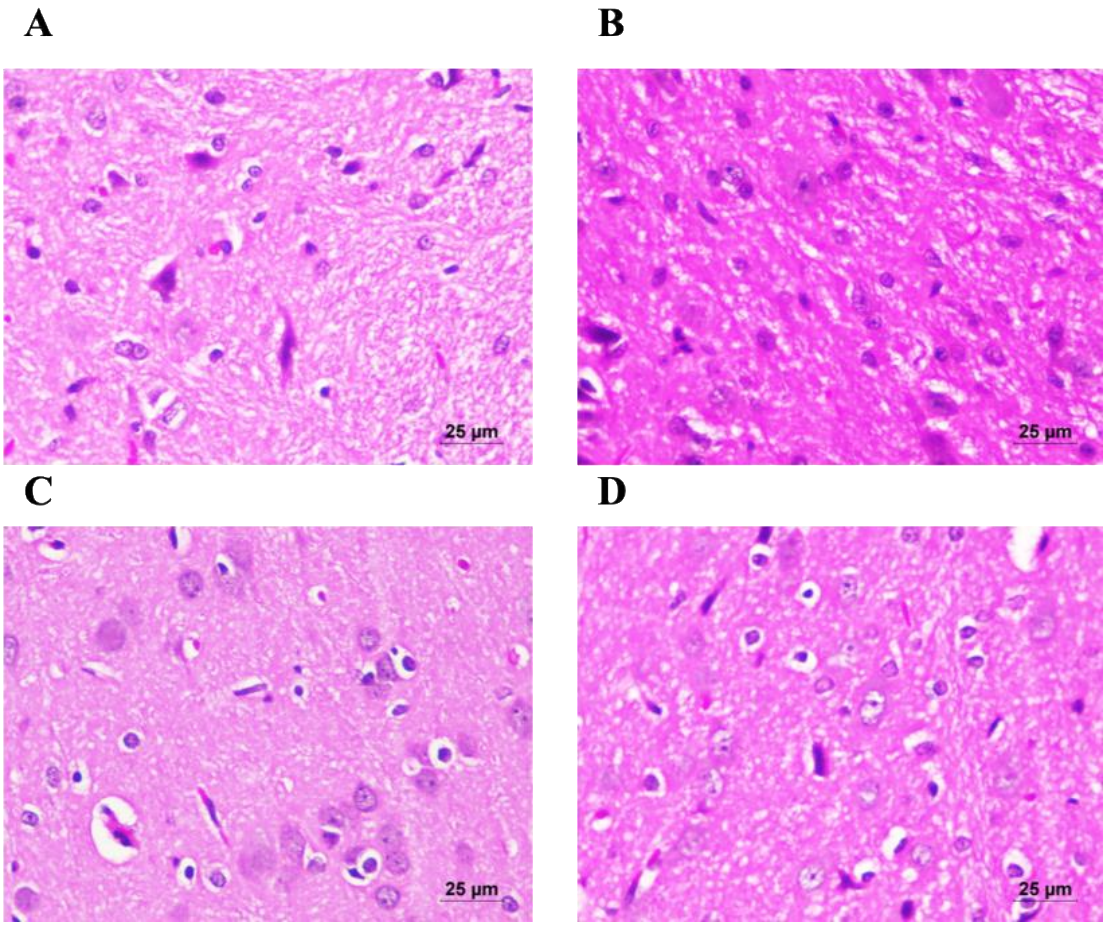

Figure 4. 60: Representative images histopathology of brain of A) Normal control male animals B) High dose treated male animal C) Normal control female animals and D) High dose treated female animal. Images are shown at 40X magnification

Representative images of tissue sections of brain are shown in Fig. 4.60. No pathological changes could be observed in brain of high dose treated animals compared with normal control of male and female animals.

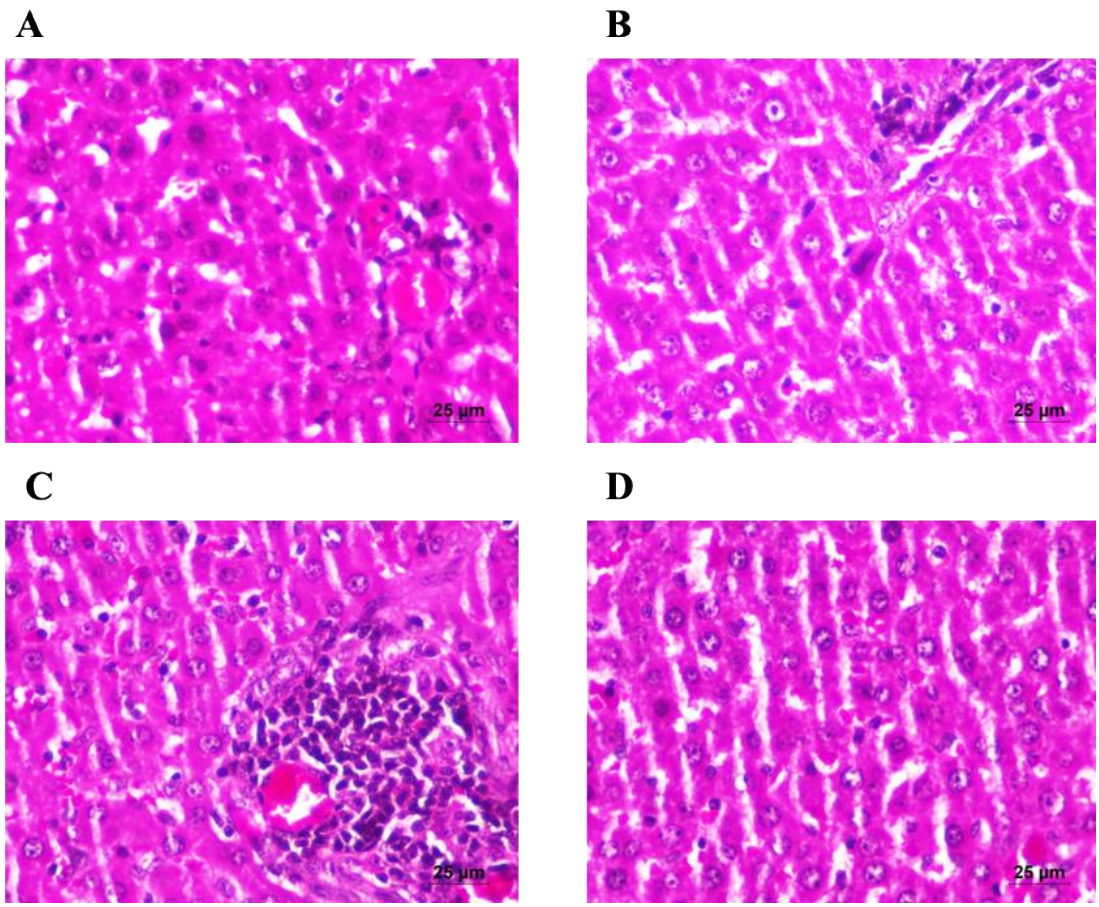

Figure 4. 61: Representative images histopathology of liver of A) Normal control male animals B) High dose treated male animal C) Normal control female animals and D) High dose treated female animal. Images are shown at 40X magnification

Representative images of tissue sections of liver are shown in Fig. 4.61. No significant abnormality in liver tissues could be noted in high dose treated animals when compared with normal control male and female animals.

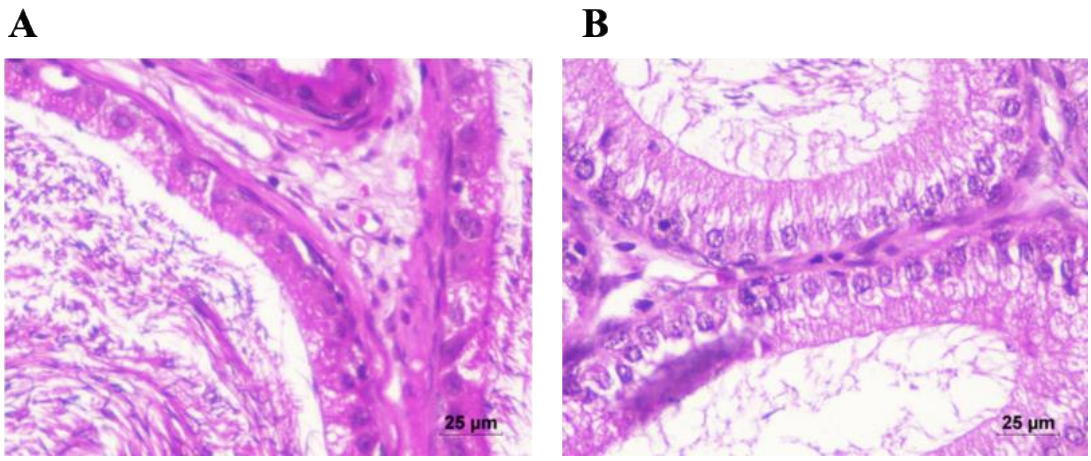

Figure 4. 62: Representative images histopathology of epididymis of A) Normal control male animals B) High dose treated male animal. Images are shown at 40X magnification

Representative images of tissue sections of epididymis are shown in Fig. 4.62. No significant abnormality in epididymis could be noted in high dose treated animals when compared with normal control male animals.

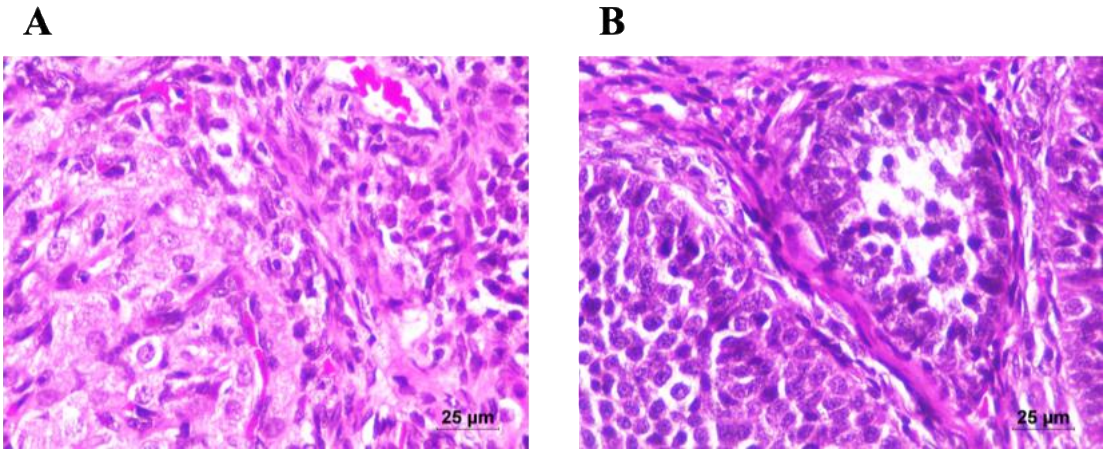

Figure 4. 63: Representative images histopathology of ovary of A) Normal control female animals B) High dose treated female animal. Images are shown at 40X magnification

Representative images of tissue sections of ovary are shown in Fig. 4.63. No significant abnormality could be noted in ovary of high dose treated animals when compared with normal control female animals.

Western blotting:

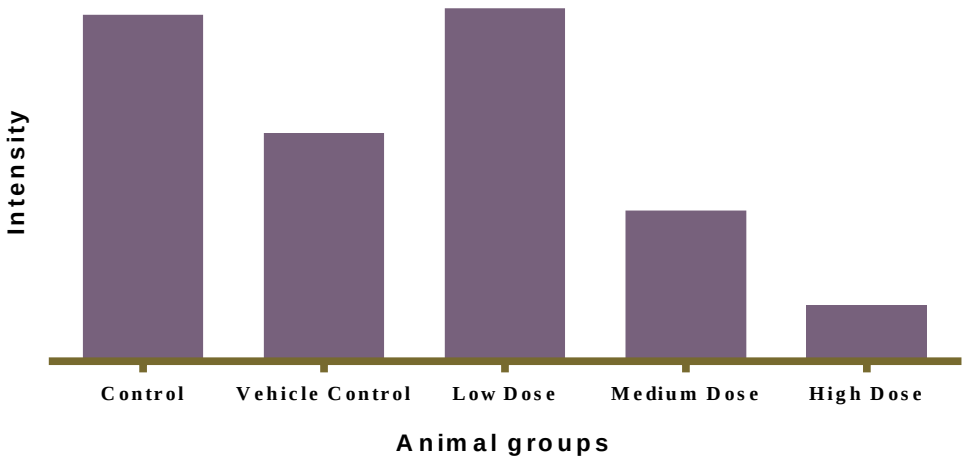

Figure 4. 64: The expression of V-CAM-1 protein in Kidney of PCM treated study animals.

The expressionof V-CAM-1 protein in kidney of the PCM treated study animals were shown in Figure.4.64. This shows that there is a decrease of V-CAM-1 protein expression in high dose treated animals.

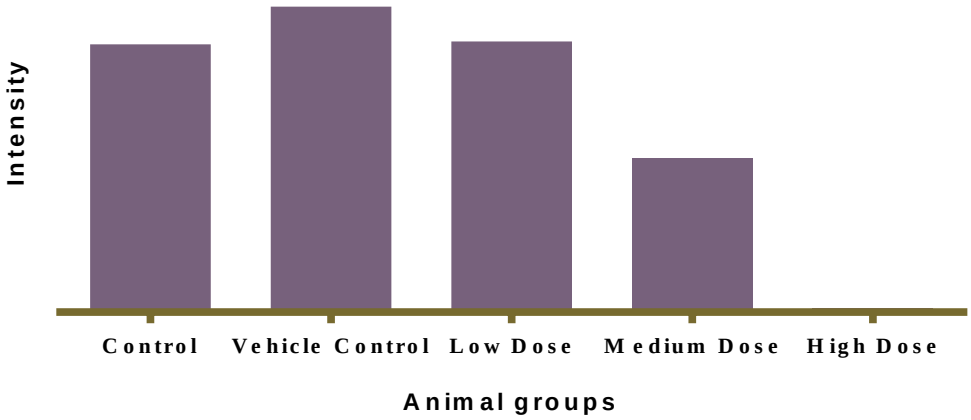

Figure 4. 65 : The expression of I-CAM-1 protein in Kidney of PCM treated study animals.

The expressionof I-CAM-1 protein in kidney of the PCM treated study animals were shown in Figure.4.65. This shows that there is a significant decrease of I-CAM-1 protein expression in high dose treated animals.

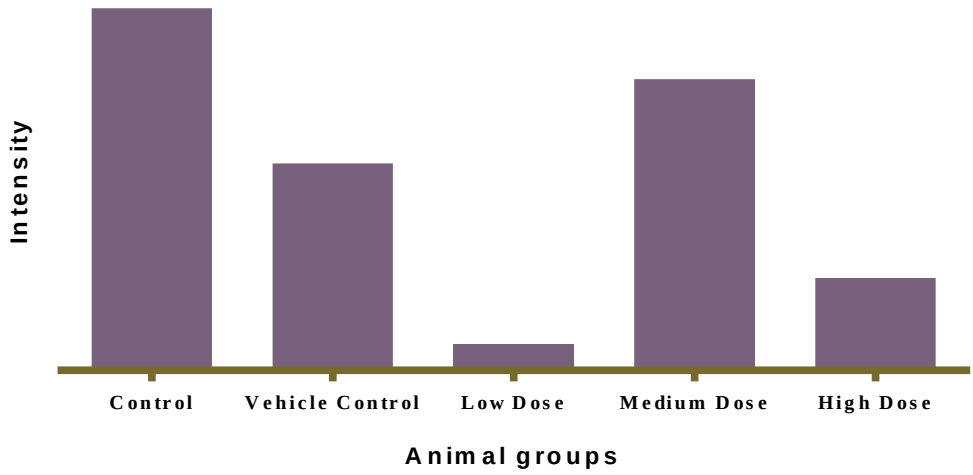

Figure 4. 66: The expression of V-CAM-1 protein in Spleen of PCM treated study animals.

The expressionof V-CAM-1 protein in Spleen of the PCM treated study animals were shown in Figure.4.66. This shows that there is a signicant decrease of V-CAM-1 protein expression in low dose treated animals.

Discussion:

The major perspective of this work is to understand the effect of PCM in short term and long term oral administration at different dose. For single oral administration, dosing was performed as per OECD guidelines. For sub-acute toxicity, animal therapeutic dose (20mg/kg) was calculated from human therapeutic dose and was fixed as low dose. Five times the animal therapeutic dose is fixed as medium dose (100mg/kg) and ten times the animal therapeutic dose (200mg/kg) is fixed as high dose.

Acute oral toxicity study:

Single oral administration of PCM produced delayed toxicity in the animals administered with dose of 550mg/kg body weight (which is more than 25 times that of therapeutic dose) and above. Mortality is seen in one of the three animals administered with 2000mg/kg Reduction in body weight and feed consumption for the first 7 days could be either due to gastric irritation or systemic inflammation. Death of one animal administered with 2000mg/kg bodyweight on the 4^{th} day indicated that, a single dose of PCM could be lethal at high doses. As the autopsy data of the dead animal is not available, the cause of death could not be clearly established. However, it could be

presumed that systemic inflammation followed by multiple organ failure could be a plausible reason as evidenced from significant loss of weight following administration. Increase in size of kidney and brain of animals administered with 2000mg/kg vouches the presence of systemic inflammation.

Sub-acute toxicity:

In sub-acute study, no significant toxicity could be observed at therapeutic doses in male and female animals. However, one mortality is noted among the 20 animals administered with five times the therapeutic dose.

Maximum number of deaths were noted on 4^{th} day with three animals surviving up to 6^{th}, 8^{th} and 10^{th} day. Previous reports on toxicity of various forms of mercury describe that oral consumption of 20mg/kg of methyl/inorganic mercury produced lethality on 2^{nd} day in male animals (75)(76)and females remain unaffected.However, in our study, PCM did not produce significant lethal effect at 20mg/kg (low dose – no mortality) and 100mg/kg (medium dose – one mortality at 8^{th} day) which signifies that the toxic effect of PCM is five times lower than that of the inorganic or organic form of mercury. Male female death ratio of 10:3, (1 medium dose, 4 high dose and 5 satellite high dose Male animals Vs 1 high dose and 2 satellite high dose animals) in mortal/moribund animals convey that males are more sensitivity to toxic effects of PCM compared with female animals. This disparity in effect of PCM between male and female rats could be ascribed to various mechanisms.The whole-body clearance of organic and inorganic mercury in female rats are proved to be faster in females than in males(77). As a consequence of this, greater developmental effects are noted in male animals than in females(78)(79)(80) proposed that the greater susceptibility of the male rat to the acute toxic effects of mercuric chloride may be due to the greater number of sulfhydryl groups in the kidneys of males as compared with females. As mercury ions have an affinity for the sulfhydryl groups of active sites in some enzymes, such as pyruvate kinase (75), a lower number of sulfhydryl groups may protect vital components of the cell such as coenzyme A from the effects of mercuric chloride (81). Quite different from Muraoka's work,*María H. Hazelhoff et al*(82), described that gender differences in the rat renal cortical Oat1 and Oat3 appear after puberty and demonstrated that the lower Oat1 and Oat3 expression in kidney from female rats restricts Hg uptake into renal cells, protecting them from this metal toxicity concluding

that Oat1 and Oat3 are among the main transporters responsible for HgCl2-induced renal injury(82).

All the animals that died in between showed piloerection, intentional tremors, paralysis, hypothermia, tip toe walking and pin point pupil before death. Presence of intentional tremors and tip toe walking can either be due to defect in the chief organs controlling co-ordination movements (cerebellum and brain stem) or could be a muscle twitching or cramping in the legs and/or arms (83) secondary to changes in electrolyte balance (i.e., potassium imbalance due to fluid loss or renal wasting). Toxic effects observed in Iraqis poisoned by consumption of mercury contaminatedfood grains resulted inloss of power in the legs, difficulty in walking, cerebellar ataxia, disturbance of speech, paraplegia, or general spasticity, ankle clonus and intentional tremors(84). Fasciculation and coarse twitching were also present. It is likely that these effects were secondary to effects on the nervous system.Though the pattern of mercury deposits by oral ingestion of inorganic mercury markedly vary from region to region within the brain, there was a clear tendency for mercury to get heavily accumulated in regionsprimarily concerned with motor systems in the brain stem and cerebellum(e.g., red nucleus, cranial nerve motor nuclei, deep nuclei of cerebellum)(85).

Mercuric ions have a very high affinity for thiol-containing biomolecules, such as glutathione (GSH), cysteine (Cys), homocysteine (Hcy), N-acetylcysteine (NAC), metallothionein (MT) and albumin(86). Hence in biological systems, they are always bound to one or more of these compounds and does not exist in an unbound ionic state (87). In 1988, Aschner and Clarkson hypothesised that organic mercury may be transported from the blood to the CNS across the blood-brain barrier by the L-type neutral amino acid carrier transport (LAT) system. A few years later, *Kerper et al*(88) observed that organic mercury entered the rat brain as a cysteine complex via the LAT system. A decade later, it was found that LAT1, was associated with increased uptake of organic mercury in the presence of L-cysteine (89). These observations culminated in the conclusion that Hg-L-cysteine conjugate is a substrate for the LAT1 system, which actively transports Hg across membranes and is at least partly responsible, for the high Hg levels found in the brain after exposures.Indeed, although other transporters have been reported to contribute to MeHg transport (90), LAT1 seems to be the main, if not the only, transporter responsible for Hg transport from peripheral tissues to the

CNS (88). As no previous studies regarding musculoskeletal effects in animals after oral exposure to inorganic mercury could be located, this could not be conclusively established.

A gradual weight loss and decrease in feed in take noted in initial stage of study (up to 8 day) in all animals (low, medium and high dose) administered with PCM rats indicates its effect in intestinal enterocytes. Ithasbeensuggestedthat the means by which mercury is transported across intestinal enterocytes depends upon the species of mercury present in the intestinal lumen, which itself is dependent upon the ligands available to which mercury can bind (91).Considering the abundance of amino acid transporters in the luminal plasma membrane of enterocytes and evidencesof amino acid and/ or peptide transporters significant roles in the intestinal absorption of mercury, it is likely that *Aṇṭatailam,* (a rich source of glutathione and other amino acids) present in PCM would have favoured the intestinal absorption of PCM. However, small amount of mercury may be taken up following ligand exchange whereby a mercuric ion is removed from its thiol carrier and is taken up bydivalent metal transporter 1 (DMT1 -An ion transporter localized in the apical membrane of enterocytes). This would have caused theaccumulation of mercury inside enterocytes (as mercury can be transported out only as a thiol conjugate) resulting in enteritis followed by decreased feed intake and reduction in body weight. Later, either due to the increased production of thiol-containing proteins due to activation of MTF genes or from external thiol sources, the accumulated mercury would have been secreted out resulting in resolving of the enteritis. As both methyl and inorganic mercury can be transported out of intestine, kidney, and probably other cells on the glutathione carrier, his molecular mimicry between mercury thiol complexes and endogenous substrates of transport carriers gives useful insights into key aspects of mercury toxicology.

Conclusion:

- In single dose oral administration, PCM did not show any lethal effect up to 550 mg/kg.
- In single dose oral administration, PCM did not show any toxicity at 175 mg/kg which is approximately more than 8 times of the therapeutic dose. Mild changes in feed consumption and body weight was noted in animal administered with 550 mg/kg.

- One among the three animals administered with 2000 mg/kg (100 times of the therapeutic dose) died on the 4thday indicating the lethal effect of PCM at very high doses.
- On repeated oral administration, PCM was found to be highly lethal at 10 times the therapeutic dose.
- Lethality was found to be 3 times higher in male compared with female possibly due to the over expression of OAT 1 and OAT 2 in kidneys or the influence of male hormones.
- Only one among the 20 animals, died on the 8th day in repeated administration of PCM at the 5 times the therapeutic dose (medium dose) signifying that PCM can be sensitive to specific individuals at medium doses.
- In all the morbid animals, neurotoxicity and musculoskeletal toxicity were observed before death signifying the toxic effect of PCM in brain at higher doses. However the confirmation can be made only after determining the mercury levels in brain tissues.
- Testicular toxicity, splenomegaly and nephrotoxicity is also noted in animals that were treated with 10 times the therapeutic dose of PCM.
- Degeneration of nephrons is observed in noted in all the animals administered with 10 times the therapeutic dose.

From this information we conclude that,

PCM is completely safe in rats at therapeutic doses in repeated oral administration for a period of 28 days in rats. Though PCM can be rarely lethal in sensitive males at the dose of 5 times that of therapeutic dose, it did not show any significant toxic effect in female animals. However, splenomegaly (that resolved within 10 days in repeated dose administration) and nephrotoxicity is observed in most of these animals. Severe neurotoxic and musculo-toxic effects were noted in all the animals that died during the course of study. A clear gender disparity is noted in lethal effects of PCM administered at 10 times the therapeutic dose with 3 times high mortality in males compared with females. Despite the neurotoxic effects observed in high dose PCM administered rats before death, survived animals did not show any neurotoxicity. Instead, nephrotoxicity is seen in most of the animals administered with high dose.The difference in toxic effects at acute stage and repeated dose administration remains unclear.

5 Summary and Conclusion

Summary and Conclusion:

1. The medicine *Pañca cūta meḻuku* (PCM) referenced in the Siddha literature *Yūkikarical*- 151 was chosen for the dissertation and approved formally by institution.
2. The metal drugs were authenticated by experts in Chemistry department, Siddha Central Research Institute, Chennai.
3. The herbal drugs were authenticated by experts in Herbal garden, Siddha Central Research Institute, Mettur dam.
4. Drug were purified as per the literature and subjected to preparatory procedures.
5. The medicine PCM was successfully prepared with precautionary measures and authenticated.
6. As per PLIM guidelines standardization parameters and qualitative analysis were studied.
7. FTIR analysis showed the presence of both organic and inorganic substances.
8. Approximately 95% degradation of PCM at with most of the mercury epaporating above 400^{o}C indicated the presence of significant organic form of mercury in final PCM
9. This was substanciated by elememtal analysis, as it showed the decrease in percentage of mercury before and after trituration.
10. Following that XRD was performed to know the speciation of elements in PCB-before trituration and PCM-FINAL and the inference indicates that the presence of $HgCl_2$ and Hg_2Cl_2 in the former but in latter HgS was the major compound.
11. One-time oral administration of PCM produced notable toxicity only at very high doses (hundred times that of therapeutic dose) and was found to be completely safe up to twenty-seven times that of therapeutic dose in female animals.

12. On continuous administration for a period of Twenty-eight days, PCM was found to be safe at therapeutic dose in male and female animals. Though mortality was seen at 8th day in one among the 10 male animals administered with five times that of therapeutic dose, female animals didn't show any lethal effects.Repeated administration of ten times that of therapeutic dose caused mortality in both male and female animals from fourth day.
13. A clear disparity is seen between male and female animals in mortality rate with male death three times more when compared with female animals. This disparity could be attributed to the influence of sex in expression of OAT 1 & OAT 2 in the renal cell membrane or direct influence of sex hormones.
14. All the animals that died during the course of study showed neurotoxic or musculo-toxic symptoms, whereas the animals that survived the complete course of study showed nephrotoxic symptoms. Upregulation of major inflammatory markers, ICAM-1 and VCAM-1 in kidney and spleen confirmed the active inflammation in both spleen and kidney of high dose administered animal

Future directions:

This study gave a primary understanding about the safety of PCM at therapeutic doses and neurotoxicity, musculotoxicity and nephrotoxicity at high doses. However, the exact organs of absorbtion, deposition and excretion was not very clear. The complete knowledge on absorbtion of PCM is possible only by performing various pharmacokinetic studies with large sample size and evaluation of few physico-chemical properties such as zeta potential in various vehicles. Moreover, a study on mercury levels in different organs after administration of PCM at various duration is essential to understand the deposition of PCM on oral exposure. Though two major proteins of inflammatory pathway is analysed in this study, the complete knowledge on effect of PCM on various signalling pathways can be obtained only performing protieomics study on various interacellular proteins (particularly cell cycle regulator proteins). Once we have all these data, we will have a complete contemporary understanding of saftety and toxic aspects of PCM.

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The Tamil Nadu Dr. M.G.R. Medical University

69, Anna Salai, Guindy, Chennai - 600 032.

This certificate is awarded to Dr/~~Mr~~/~~Mrs~~...R. JEEVANANDHINI.....

for participating as ~~Resource~~ ~~Person~~ / Delegate in the XXIII Workshop on

"RESEARCH METHODOLOGY & BIOSTATISTICS"

Organized by the Department of Siddha,

The Tamil Nadu Dr. M.G.R. Medical University from 6th to 10th March 2017.

Dr. N. KABILAN, M.D.(Siddha)
PROF & HEAD
Dept of Siddha

Dr. T.BALASUBRAMANIAN M.S.,D.L.O.,
REGISTRAR

Prof. Dr. S.GEETHALAKSHMI, M.D.,Ph.D.,
VICE CHANCELLOR

GOVT.SIDDHA MEDICAL COLLEGE

PALAYAMKOTTAI

SCREENING COMMITTEE

Candidate reg.no : ...32161600 2.....

Department : Nanju Noolum Maruthuva Neethi Noolum.

This is to certify that the dissertation topic **A Toxicity study on"PANCHA SOOTHA MEZHUGU"**has been approved by the screening committee.

Branch	Department	Name	Signature
1	PothuMaruthuvam	Dr.A.Manoharan. MD(S)., Professor	
2	Gunapadam	Dr.A.KingslyMD(S)., Associate Professor	
3	SirappuMaruthuvam	Dr.A.S.PoongodikanthimathiMD(S)., Professor	
4	KuzhandhaiMaruthuvam	Dr.D.K.Soundararajan. MD(S)., Professor	
5	NoiNadal	Dr.S.VictoriaMD(S)., Professor	
6	NanjuNoolMaruthuvam	Dr.M.Thiruthani. MD(S)., Professor	

Remarks:

PRINCIPAL
Govt. Siddha Medical College
Palayamkottai.

சித்த மருத்துவ மூலிகைத் தோட்டம்
(மத்திய சித்தமருத்துவ ஆராய்ச்சிக் குழுமம்)
(ஆயுஷ் அமைச்சகம், இந்திய அரசு)
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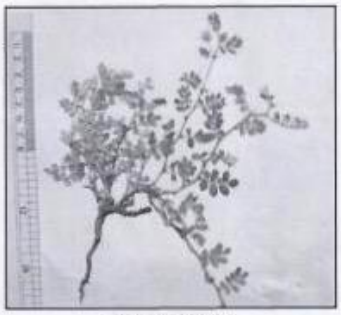
No. 17, SDO Quarters, Opp. Ulavar Santhai, Cauvery Nagar, MetturDam, Tamilnadu-636 401
Phone No. 04298 – 243 773 E-mail:smpgmettur@gmail.com

Date: 17/6/19

AUTHENTICATION CERTIFICATE FOR 170619006

Certified that the drug submitted by **Dr. R.Jeevanandhini, 3rd Year PG (Reg. No.321616002)** Department of Nanju Noolum Maruthuva Neethi Noolum, Govt. Siddha Medical College and Hospital, Palayamkottai is identified as:

S.No	Botanical Name/Family	Tamil Name	Part	Code
1	*Tribulus terrestris* L./ Zygophyllaceae	Nerunjil	Whole plant	T170619006T



T170619006T

17/6/19.

Dr. P. Radha
Research Officer (Botany), I/C

RESEARCH OFFICER - Botany I/C
Siddha Medicinal Plants Garden,
(CCRS, Govt. Of India)
Mettur Dam - 636 401.

சித்த மருத்துவ மூலிகைத் தோட்டம்
(மத்திய சித்தமருத்துவ ஆராய்ச்சிக் குழுமம்)
(ஆயுஷ் அமைச்சகம், இந்திய அரசு)
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Phone No. 04298 – 243 773 E-mail:smpgmettur@gmail.com

Date: 19/6/19

AUTHENTICATION CERTIFICATE FOR 170619005

Certified that the drug submitted by **Dr. R.Jeevanandhini, 3rd Year PG (Reg. No.321616002)** Department of Nanju Noolum Maruthuva Neethi Noolum, Govt. Siddha Medical College and Hospital, Palayamkottai is identified as:

S.No	Botanical Name/Family	Tamil Name	Part	Code
1	*Moringa oleifera* Lam./ Moringaceae	Murungai	Flower	M170619005O

M170619005O

19/6/19

Dr. P. Radha
Research Officer (Botany), I/C
RESEARCH OFFICER - Botany I/C
Siddha Medicinal Plants Garden,
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Mettur Dam - 636 401.

சித்தமருத்துவ மைய ஆராய்ச்சி நிலையம்
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सिद्ध केंद्रीय अनुसन्धान संस्थान
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SCRI-Tech./2019-20/Ext. Samples/81/AMDRL/16 10th July 2019

To

Dr.R.Jeevanandhini
Postgraduate Scholar,
Department of Nanju Maruthuvam,
Govt Siddha Medical Collage,
Palayamkottai

Sub: Authentication certificate - reg

Sir,

Find the authentication certificates of sample namely Pooram, submitted to Animal & Mineral origin Drug Research Laboratory (AMDRL) of Siddha Central Research Institute, Chennai.

डॉ. पी. सत्तियराजेश्वरन/Dr.P.Sathiyarajeswaran
प्रभारी सहायक निदेशक (एस-II)/Assistant Director (S-II) I/C
सिद्ध केंद्रीय अनुसंधान संस्थान
(केन्द्रीय सिद्ध अनुसंधान परिषद, आयुष मंत्रालय, भारत सरकार)
अन्ना सरकारी अस्पताल परिसर, अरुम्बाक्कम, चेन्नई-600 106
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Anna Govt. Hospital Campus, Arumbakkam, Chennai 600106

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F.No.: **SCRI/AMDRL/2019-20/ICP-OES/23** 10-07-2019

AUTHENTICATION CERTIFICATE

Samples Name : Pooram
Sample submitted by : Dr.R.Jeevanandhini
Institutional details : Postgraduate Scholar,
Department of Nanju Maruthuvam,
Govt Siddha Medical Collage,
Palayamkottai

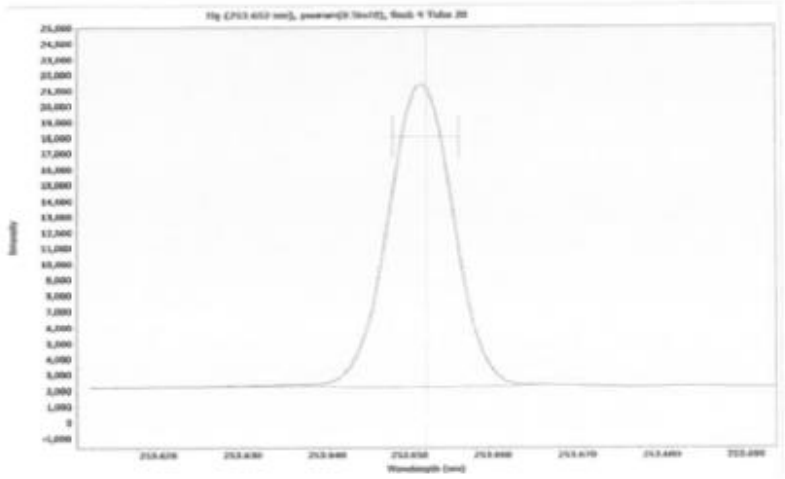
It is certified that the sample ,Pooram, is identified as "Mercurous Chloride" based on the identification test by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES). The spectrum of Mercury at 253.652 nm was studied.

Procedure:

Take about 50 mg of sample into the Teflon microwave digestion vessel and add 1 mL of ultrapure nitric acid to digest about 45 minutes using Anton Paar microwave digestion unit. After that the sample is made up to a 50 mL standard measuring flask. The calibration standard solution is prepared for 0.25 µg/mL to 10 µg/mL by using ultrapure nitric acid and blank also. Agilent ICP-OES 5100 VDV instrument used with the following operation conditions: a RF power 1.2 kW, a plasma gas flow rate 12 L min^{-1}, and a nebulizer gas flow rate 0.70 L min^{-1}. The samples are introduced into the plasma using nebulizer and spray chamber for the analysis of the element.

Research Officer (Chemistry)
सिद्ध केंद्रीय अनुसंधान संस्थान/Siddha Central Research Institute

ICP-OES Report



ICP-OE spectrum of Mercury in Pooram

End of the report-

ICP-OES Report

சித்தமருத்துவ மைய ஆராய்ச்சி நிலையம்
(மத்திய சித்த மருத்துவ ஆராய்ச்சிக் குழுமம், ஆயுஷ் அமைச்சகம், இந்திய அரசு)
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SCRI-Tech./2019-20/Ext. Samples/81/AMDRL/15 10th July 2019

To

Dr.R.Jeevanandhini
Postgraduate Scholar,
Department of Nanju Maruthuvam,
Govt Siddha Medical Collage,
Palayamkottai

Sub: Authentication certificate - reg

Sir,

Find the authentication certificates of sample namely Veeram, submitted to Animal & Mineral origin Drug Research Laboratory (AMDRL) of Siddha Central Research Institute, Chennai.

डॉ. पी. शनियराजेश्वरन/Dr.P.Shaniyarajeswaran
सहायक निदेशक (एस-II)/Assistant Director (S-II) I/C
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Anna Govt. Hospital Campus, Arumbakkam, Chennai 600106

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F.No.: **SCRI/AMDRL/2019-20/ICP-OES/22** 10-07-2019

AUTHENTICATION CERTIFICATE

Samples Name : Veeram

Sample submitted by : Dr.R.Jeevanandhini

Institutional details : Postgraduate Scholar,
Department of Nanju Maruthuvam,
Govt Siddha Medical Collage,
Palayamkottai

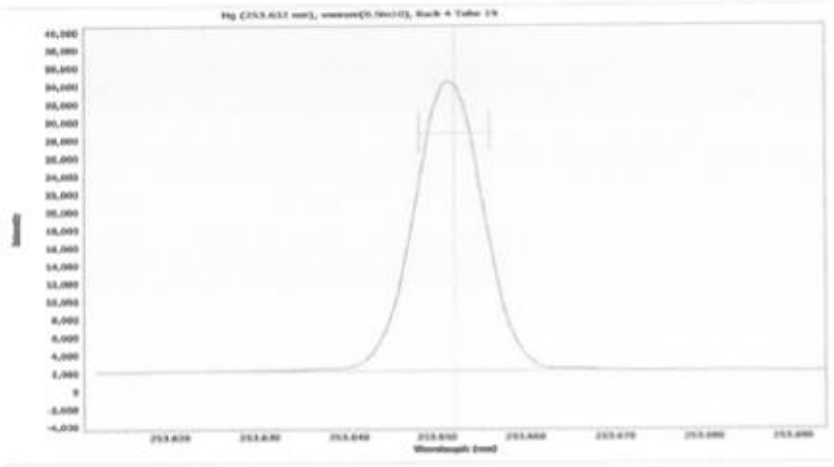
It is certified that the sample veeram, is identified as "Mercury Chloride" based on the identification test by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES). The spectrum of Mercury at 253.652 nm was studied.

Procedure:

Take about 50 mg of sample into the Teflon microwave digestion vessel and add 1 mL of ultrapure nitric acid to digest about 45 minutes using Anton Paar microwave digestion unit. After that the sample is made up to a 50 mL standard measuring flask. The calibration standard solution is prepared for 0.25 µg/mL to 10 µg/mL by using ultrapure nitric acid and blank also. Agilent ICP-OES 5100 VDV instrument used with the following operation conditions: a RF power 1.2 kW, a plasma gas flow rate 12 L min^{-1}, and a nebulizer gas flow rate 0.70 L min^{-1}. The samples are introduced into the plasma using nebulizer and spray chamber for the analysis of the element.

Research Officer (Chemistry)

ICP-OES Report



ICP-OE spectrum of Mercury in Veeram

-End of the report-

ICP-OES Report

சித்தமருத்துவ மைய ஆராய்ச்சி நிலையம்
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SCRI-Tech./2019-20/Ext. Samples/81/AMDRL/18 10th July 2019

To

Dr.R.Jeevanandhini
Postgraduate Scholar,
Department of Nanju Maruthuvam,
Govt Siddha Medical Collage,
Palayamkottai

Sub: Authentication certificate - reg

Sir,

Find the authentication certificates of sample namely Lingam, submitted to Animal & Mineral origin Drug Research Laboratory (AMDRL) of Siddha Central Research Institute, Chennai.

डॉ. पी. सतियराजेश्वरन / Dr.P.Sathiyarajeswaran
सहायक निदेशक (एस-II) / Assistant Director (S-II) I/C
सिद्ध केंद्रीय अनुसंधान संस्थान,
(केन्द्रीय सिद्ध अनुसंधान परिषद, आयुष मंत्रालय, भारत सरकार)
अण्णा सरकारी अस्पताल परिसर, अरुम्बाक्कम, चेन्नई-600 106
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F.No.: **SCRI/AMDRL/2019-20/ICP-OES/25** 10-07-2019

AUTHENTICATION CERTIFICATE

Samples Name : Lingam

Sample submitted by : Dr.R.Jeevanandhini

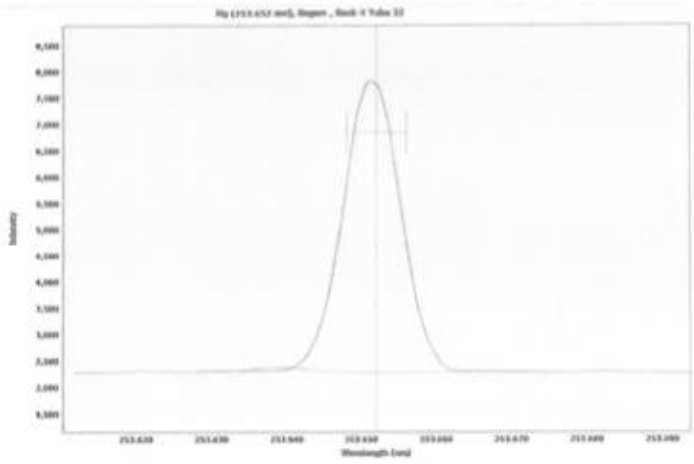
Institutional details : Postgraduate Scholar,
Department of Nanju Maruthuvam,
Govt Siddha Medical Collage,
Palayamkottai

It is certified that the sample ,Lingam is identified as "Red Sulfide of Mercury" based on the identification test by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES). The spectrum of Mercury at 253.652 nm was studied.

Procedure:

Take about 50 mg of sample into the Teflon microwave digestion vessel and add 1 mL of ultrapure nitric acid to digest about 45 minutes using Anton Paar microwave digestion unit. After that the sample is made up to a 50 mL standard measuring flask. The calibration standard solution is prepared for 0.25 µg/mL to 10 µg/mL by using ultrapure nitric acid and blank also. Agilent ICP-OES 5100 VDV instrument used with the following operation conditions: a RF power 1.2 kW, a plasma gas flow rate 12 L min^{-1}, and a nebulizer gas flow rate 0.70 L min^{-1}. The samples are introduced into the plasma using nebulizer and spray chamber for the analysis of the element.

अनुसंधान अधिकारी (रसायन)
Research Officer (Chemistry)
सिद्ध केन्द्रीय अनुसंधान संस्थान/Siddha Central Research Institute
केन्द्रीय सिद्ध अनुसंधान परिषद/Central Council for Research in Siddha
आयुष मंत्रालय, भारत सरकार/Ministry of AYUSH, Govt. of India
अरुम्बाक्कम, चेन्नै-६०० १०६/Arumbakkam, Chennai - 600 106



ICP-OE spectrum of Mercury in Lingam

End of the report-

ICP-OES Report

சித்தமருத்துவ மைய ஆராய்ச்சி நிலையம்
(மத்திய சித்த மருத்துவ ஆராய்ச்சிக் குழுமம், ஆயுஷ் அமைச்சகம், இந்திய அரசு)
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(सी.सी.आर.एस., चेन्नई, आयुष मंत्रालय, भारत सरकार), अण्णा सरकारी अस्पताल परिसर, अरुम्बाक्कम, चेन्नई - 600106
SIDDHA CENTRAL RESEARCH INSTITUTE
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Anna Govt. Hospital Campus, Arumbakkam, Chennai – 600106, E-mail: crisiddha@gmail.com
Phone: 044-26214925, 26214809, Web: http://crisiddha.tn.nic.in

SCRI-Tech./2019-20/Ext. Samples/81/AMDRL/17 10th July 2019

To

Dr.R.Jeevanandhini
Postgraduate Scholar,
Department of Nanju Maruthuvam,
Govt Siddha Medical Collage,
Palayamkottai

Sub: Authentication certificate - reg

Sir,

Find the authentication certificates of sample namely Rasa Sindooram, submitted to Animal & Mineral origin Drug Research Laboratory (AMDRL) of Siddha Central Research Institute, Chennai.

डॉ. पी. सत्यराजेश्वरन / Dr. P. Sathyarajeswaran
प्रभारी सहायक निदेशक (ग्रे-II)/Assistant Director (S-II) I/C
सिद्ध केंद्रीय अनुसंधान संस्थान
(केंद्रीय सिद्ध अनुसंधान परिषद, आयुष मंत्रालय, भारत सरकार)
अण्णा सरकारी अस्पताल परिसर, अरुम्बाक्कम, चेन्नई-600 106
SIDDHA CENTRAL RESEARCH INSTITUTE
(Central Council for Research in Siddha, Ministry of AYUSH, Govt. of India)
Anna Govt. Hospital Campus, Arumbakkam, Chennai 600106

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F.No.: **SCRI/AMDRL/2019-20/ICP-OES/24** 10-07-2019

AUTHENTICATION CERTIFICATE

Samples Name : Rasa sindooram

Sample submitted by : Dr.R.Jeevanandhini

Institutional details : Postgraduate Scholar,
Department of Nanju Maruthuvam,
Govt Siddha Medical Collage,
Palayamkottai

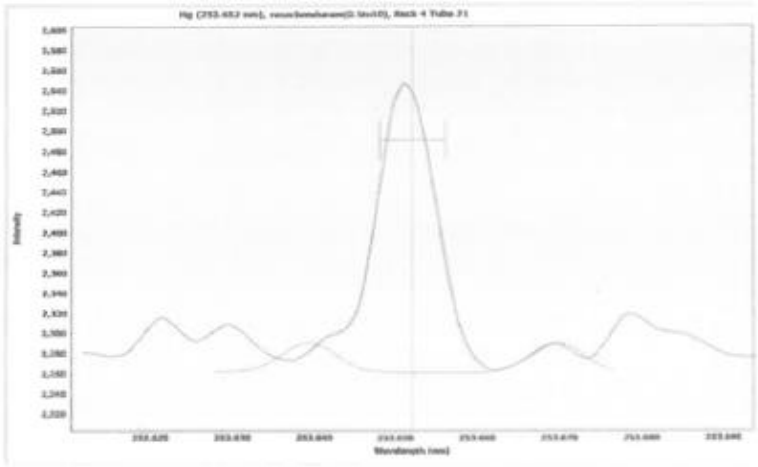
It is certified that the sample ,Rasa sindooram is identified as "Red Sulfide of mercury-Artifical" based on the identification test by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES). The spectrum of Mercury at 253.652 nm was studied.

Procedure:

Take about 50 mg of sample into the Teflon microwave digestion vessel and add 1 mL of ultrapure nitric acid to digest about 45 minutes using Anton Paar microwave digestion unit. After that the sample is made up to a 50 mL standard measuring flask. The calibration standard solution is prepared for 0.25 µg/mL to 10 µg/mL by using ultrapure nitric acid and blank also. Agilent ICP-OES 5100 VDV instrument used with the following operation conditions: a RF power 1.2 kW, a plasma gas flow rate 12 L min^{-1}, and a nebulizer gas flow rate 0.70 L min^{-1}. The samples are introduced into the plasma using nebulizer and spray chamber for the analysis of the element.

अनुसंधान अधिकारी (रसायन)
Research Officer (Chemistry)
सिद्ध केंद्रीय अनुसंधान संस्थान/Siddha Central Research Institute
केंद्रीय सिद्ध अनुसंधान परिषद/Central Council for Research in Siddha
आयुष मंत्रालय, भारत सरकार/Ministry of AYUSH, Govt. of India
अरुम्बाक्कम, चेन्नै-६०० १०६./Arumbakkam, Chennai - 600 106.

ICP-OES Report



ICP-OE spectrum of Mercury in Rasa sindooram

End of the report-

ICP-OES Report

சித்தமருத்துவ மைய ஆராய்ச்சி நிலையம்
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SCRI-Tech./2019-20/Ext. Samples/81/AMDRL/19 10th July 2019

To

Dr.R.Jeevanandhini
Postgraduate Scholar,
Department of Nanju Maruthuvam,
Govt Siddha Medical Collage,
Palayamkottai

Sub: Authentication certificate - reg

Sir,

Find the authentication certificates of sample namely Rasam, submitted to Animal & Mineral origin Drug Research Laboratory (AMDRL) of Siddha Central Research Institute, Chennai.

डॉ. पी. सत्यराजेश्वरन / Dr. P. Sathyarajeswaran
प्रभारी सहायक निदेशक (ग्रे-II) / Assistant Director (S-II) I/C
सिद्ध केंद्रीय अनुसंधान संस्थान,
(केन्द्रीय सिद्ध अनुसंधान परिषद, आयुष मंत्रालय, भारत सरकार)
अन्ना सरकारी अस्पताल परिसर, अरुम्बाक्कम, चेन्नई-600 106
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F.No.: **SCRI/AMDRL/2019-20/ICP-OES/26** 10-07-2019

AUTHENTICATION CERTIFICATE

Samples Name : Rasam

Sample submitted by : Dr.R.Jeevanandhini

Institutional details : Postgraduate Scholar,
Department of Nanju Maruthuvam,
Govt Siddha Medical Collage,
Palayamkottai

It is certified that the sample ,Rasam is identified as "Mercury" based on the identification test by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES). The spectrum of Mercury at 253.652 nm was studied.

Procedure:

Take about 50 mg of sample into the Teflon microwave digestion vessel and add 1 mL of ultrapure nitric acid to digest about 45 minutes using Anton Paar microwave digestion unit. After that the sample is made up to a 50 mL standard measuring flask. The calibration standard solution is prepared for 0.25 µg/mL to 10 µg/mL by using ultrapure nitric acid and blank also. Agilent ICP-OES 5100 VDV instrument used with the following operation conditions: a RF power 1.2 kW, a plasma gas flow rate 12 L min^{-1}, and a nebulizer gas flow rate 0.70 L min^{-1}. The samples are introduced into the plasma using nebulizer and spray chamber for the analysis of the element.

अनुसंधान अधिकारी (रसायन)
Research Officer (Chemistry)
सिद्ध केंद्रीय अनुसंधान संस्थान/Siddha Central Research Institute
केंद्रीय सिद्ध अनुसंधान परिषद/Central Council for Research in Siddha
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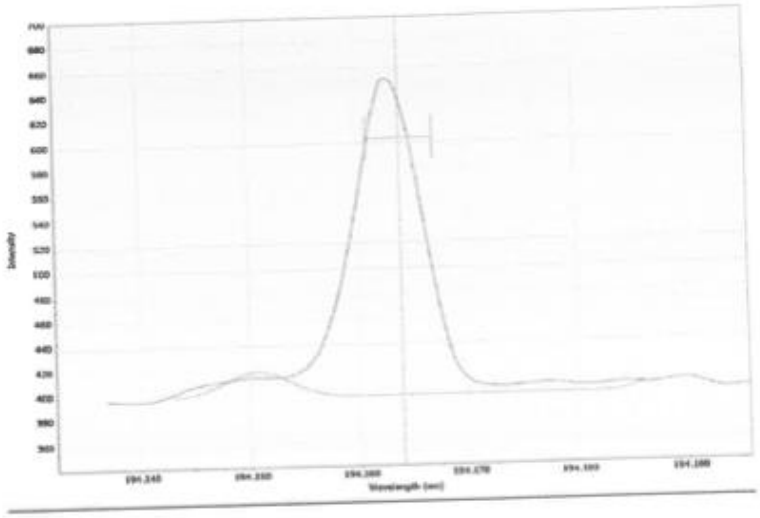
ICP-OES Report



ICP-OE spectrum of Mercury in Rasam

End of the report-

ICP-OES Report

SASTRA
DEEMED TO BE UNIVERSITY
(U/S 3 OF THE UGC ACT, 1956)
THANJAVUR - 613 401, INDIA

CERTIFICATE

This is to certify that the project entitled, Evaluation of acute and sub-acute oral toxicity of Pañca cūta mel̤uku on Wistar rats

has been approved by the Institutional Animal Ethics Committee (IAEC), SASTRA Deemed University held on 06.01.2019

CPCSEA Approval Number: 590/SASTRA/IAEC/EPP

Chairman's Signature 06/01/19

Nominee's Signature 06/01/19

Name of Chairman / Member Secretary, IAEC: Dr. C David Raj

Name of CPCSEA Nominee: Dr. V. Goputhaman

GOVERNMENT SIDDHA MEDICAL COLLEGE& HOSPITAL
PALAYAMKOTTAI

CME PROGRAMME

Conducted by
SIRAPPU MARUTHUVAM
DEPARTMENT
GSMCH - PALAYAMKOTTAI

Organised by
ASHTAVAIDYAN THAIKKATTU MOOSS
VAIDYARATNAM

Supported by

S.No: 093

CERTIFICATE

This Certifies that
Dr. R. Jeeva Nandhini
has participated in Continuing Medical Education on "AYUSH External Therapies-II" held at GSMCH, Palayamkottai on Dec, 4 2018

Dr. A.S.Poongodi Kanthimathi MD (s)..
Head - Dept. of Sirappu Maruthuvam

Authorised Signatory
VAIDYARATNAM

Dr. R. Neelavathy MD (s), Ph.D.,
Principal

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